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(54) Title: RETROVIRAL PROTEASE INHIBITOR COMBINATIONS

(57) Abstract

The present invention is directed to a method for the treatment of mammalian retrovirus infections, such as HIV, using combinations of retroviral protease inhibitors which are effective in preventing the replication of the retroviruses *in vitro* or *in vivo*. This invention, in particular, relates to protease inhibitor compounds used in combination therapy with other protease inhibitor compounds. This invention also relates to combination therapy with a combination of protease inhibitors and antiviral agents other than protease inhibitors.

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RETROVIRAL PROTEASE INHIBITOR COMBINATIONS

BACKGROUND OF THE INVENTION

5

1. Field of the Invention

The present invention relates to a method for the treatment of mammalian retrovirus infections, such as human
10   immunodeficiency virus (HIV), using combinations of retroviral protease inhibitors which are effective in preventing the replication of mammalian retroviruses, like HIV, in vitro and in vivo. This invention, in particular, relates to protease inhibitor compounds used in combination
15   therapy with other protease inhibitor compounds.

2. Related Art

During the replication cycle of retroviruses, gag and
20   gag-pol gene transcription products are translated as proteins. The proteins are subsequently processed by a virally encoded protease (or proteinase) to yield viral enzymes and structural proteins of the virus core. Most commonly, the gag precursor proteins are processed into the
25   core proteins and the pol precursor proteins are processed into the viral enzymes, e.g., reverse transcriptase and retroviral protease. It has been shown that correct processing of the precursor proteins by the retroviral protease is necessary for assembly of infectious virons.
30   For example, it has been shown that frameshift mutations in the protease region of the pol gene of HIV prevents processing of the gag precursor protein. It has also been shown through site-directed mutagenesis of an aspartic acid residue in the HIV protease active site that processing of
35   the gag precursor protein is prevented. Thus, attempts have

been made to inhibit viral replication by inhibiting the action of retroviral proteases.

Retroviral protease inhibition typically involves a transition-state mimetic whereby the retroviral protease is
5 exposed to a mimetic compound which binds (typically in a reversible manner) to the enzyme in competition with the gag and gag-pol proteins to thereby inhibit specific processing of structural proteins and the release of retroviral protease itself. In this manner, retroviral replication
10 proteases can be effectively inhibited.

Several classes of mimetic compounds have been proposed, particularly for inhibition of proteases, such as for inhibition of HIV protease. Such mimetics include hydroxyethylamine isosteres, reduced amide isosteres and
15 non-peptide isosteres. See for example, EP 0 346 847; EP 0 342 541; Roberts et al, "Rational Design of Peptide-Based Proteinase Inhibitors," Science, 248, 358 (1990); Erickson et al, "Design Activity, and 2.8Å Crystal Structure of a C_2 Symmetric Inhibitor Complexed to HIV-1 Protease," Science,
20 249, 527 (1990); and S. Thaisrivongs, "Structure-Based Design of Non-Peptide HIV Protease Inhibitors," 35th Annual Buffalo Medicinal Chemistry Meeting, State University of New York at Buffalo, Buffalo, NY, May 22-25, 1994.

A problem for retroviral protease inhibitors, like HIV
25 protease inhibitors, has been the development of strains of the virus resistant to the inhibitor. For example, Merck & Co.'s HIV protease inhibitor L-735,524 is effective against HIV infections in humans, but L-735,524 resistant strains of HIV later develop in patients (Waldholz, The Wall Street
30 Journal, February 25, 1994, page B3; and Condra et al., Nature 374:569-571 (1995)). Other examples can be found in Vacca et al., Proc. Natl. Acad. Sci. USA 91:4096-4100 (1994); Ho et al., J. Virol. 68:2016-2020 (1994); and Sardana et al., Biochem. 33:2004-2010 (1994).

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BRIEF DESCRIPTION OF THE INVENTION

The present invention is directed to a method for the
treatment of mammalian retrovirus infections, such as human
5   immunodeficiency virus (HIV), using combinations of
retroviral protease inhibitors which are effective in
preventing the replication of the retroviruses in vitro or
in vivo. This invention, in particular, relates to protease
inhibitor compounds used in combination therapy with other
10   protease inhibitor compounds. Further, this combination may
also be used in combination with other anti-viral agents.

DETAILED DESCRIPTION OF THE INVENTION

15   Retroviral protease is a critical enzyme in the
retroviral replication process. Propagation of a
retrovirus, such as HIV, can be impeded by exposing the
virus to a retroviral protease inhibitor. However, with
prolonged exposure of the retrovirus to the protease
20   inhibitor, the variant retroviruses can be selected such
that a new predominant strain of retrovirus resistant to the
protease inhibitor emerges. The new predominant strain of
retrovirus can produce a protease which is no longer
inhibited or more frequently is insufficiently inhibited by
25   the protease inhibitor and can freely propagate even in the
presence of the protease inhibitor unless the concentration
of the inhibitor is substantially increased. The present
invention provides a method for overcoming the development
of retroviral strains which are resistant to a retroviral
30   protease inhibitor.
The present method provides for the administration to a
mammal, such as a human, monkey, cat and the like, of an
effective amount of at least two retroviral protease
inhibitors. The administration may be accomplished by co-
35   administration of at least two retroviral protease

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inhibitors, i.e., administering two or more retroviral
protease inhibitors such that an effective amount of at
least two inhibitors are present in said mammal at any one
time. Alternatively, the administration may be accomplished
5   by sequential or alternating administration of at least two
retroviral protease inhibitors, i.e. administering two or
more retroviral protease inhibitors such that an effective
amount of only one inhibitor is present in said mammal at
any one time. With the proper selection of the retroviral
10   protease inhibitors, this method can effectively control the
propagation of the retrovirus even in the presence of
resistant strains to any one of the inhibitors.
        The retroviral protease inhibitors are selected based
on the profile of resistant strain(s) of the retrovirus
15   which emerge in vivo or in vitro upon exposure of the
inhibitor to a propagating culture of the retroviruses. The
retroviral protease inhibitors are selected for lack of
cross-resistance by at least one retroviral resistant
strain. A retroviral strain is considered to be cross-
20   resistant to two protease inhibitors when the retroviral
strain is resistant to both inhibitors. While some cross-
resistance can be tolerated, preferably, no cross-resistance
exists between the selected retroviral protease inhibitors
when taken as a group. Thus a variant (or mutant strain) of
25   retrovirus which may develop as a result of exposure to a
first retroviral protease inhibitor would still be inhibited
by a second retroviral protease inhibitor or which may
develop as a result of exposure to both a first and second
retroviral protease inhibitors would still be inhibited by a
30   third retroviral protease inhibitor or a fourth retroviral
protease inhibitor and so forth.
        A comparison of cross-resistance profiles between
various protease inhibitors are made and compounds are
selected for combination therapy that preferably exhibit
35   little or no cross-resistance. The drug resistance

phenotype may be divided into no resistance, low level
resistance (less than about 10 fold shift in EC_{50} or EC_{90}),
intermediate level resistance (about 10 to about 100 fold
shift in EC_{50} or EC_{90}) or high level resistance (greater

5   than about 100 fold shift in EC_{50} or EC_{90}). It is
anticipated that drug resistance will correlate with a
reduced effect on patient viral load when the achievable in
vivo inhibitor concentrations have a reduced protease
inhibition effect on the resistant virus. Thus the more

10 preferred combinations of protease inhibitors will be those
that exhibit minimal cross-resistance profiles (i.e.,
preferably, not more than intermediate level resistance;
more preferably, not more than low level resistance; and
most preferably, no resistance) and maximal intrinsic

15 potency for wild-type and/or resistant viruses selected
against another inhibitor. For example, preferred compounds
for use in combination with a first compound will preferably
be effective against strains of virus which are intermediate
level, more preferably high level, resistant to the first

20 compound. The pharmacology and toxicology of each inhibitor
and combination are also factors in the selection of
inhibitors for combination therapy.

More preferably, retroviral protease inhibitors are
chosen when at least one viral resistant strain to a first

25 retroviral protease inhibitor and at least one viral
resistant strain to a second retroviral protease inhibitor
having different amino acid substitutions in the protease
peptide sequence that affect the same substrate binding site
region of the protease and contributes to the observed

30 inhibitor resistance. Thus the number of possible amino
acid substitutions that may occur in the same site in the
protease are limited. This is particularly true when the
site is critical to activity, effectiveness and/or stability
of the enzyme.

This event was observed in relation to the HIV protease inhibitors of Examples 1 and 2 hereof. Retroviral resistance to the compound of Example 1 resulted from site mutation at amino acid 88 of HIV protease (substitution of

5   asparagine 88 with aspartic acid 88). Retroviral resistance to the compound of Example 2 also resulted from site mutation at amino acid 88 of HIV protease (substitution of asparagine 88 with serine 88). Some substitutions at amino acid 88 are known to cause loss of enzyme activity (Loeb et

10 al., Nature 340:387-400 (1989)). Thus the administration of both HIV protease inhibitors of Examples 1 and 2 substantially reduces the likelihood of further successful production of a resistant strain of virus cross-resistant to both inhibitors. No resistance to both inhibitors used in

15 combination has been detected through 6 weeks of treatment compared to the emergence of a resistant phenotype to a single inhibitor in the same time frame. In addition to site mutations which affect enzyme activity, other site mutations in the same variant may also arise which do not

20 substantially affect enzyme activity and/or resistance.

Alternatively, more preferably, retroviral protease inhibitors are selected when at least one viral resistant strain to a first retroviral protease inhibitor has an increased sensitivity to said second protease inhibitor or

25 when at least one viral resistant strain to a second retroviral protease inhibitor has an increased sensitivity to said first protease inhibitor.

Representative retroviral protease inhibitors which are suitable for use in the present method include, but are not

30 limited to, the protease inhibitors disclosed and described in co-owned and co-pending US Patent Applications Serial Nos. 08/152,934 (filed November 15, 1993), 08/253,531 (filed June 3, 1994), 08/109,787 (filed August 20, 1993), 08/110,911 (filed August 24, 1993), 08/110,913 (filed August

35 24, 1993, 08/110,912 (filed August 24, 1993), 08/204,827

(filed March 2, 1994), 07/886,556 (filed May 20, 1992),
07/886,663 (filed May 20, 1992), 07/886,531 (filed May 20,
1992), 08/148,817 (filed November 8, 1993), 08/886,700
(filed May 21, 1992) and 07/998,187 (filed December 29,

5   1992) and PCT Patent Applications Nos. PCT/US93/10552 (filed
October 29, 1993), PCT/US93/10460 (filed October 29, 1993)
and PCT/US93/10461 (filed October 29, 1993), each of which
are incorporated herein by reference in their entirety.
Additional retroviral protease inhibitors which are suitable

10   for use in the present method include, but are not limited
to, the protease inhibitors disclosed and described in US
Patent 5,157,041; EP 346,847; US Patent Application Serial
No. 07/883,825 (filed May 15, 1992); WO 93/09096; Tet. Lett.
35:673-676 (1994); Proc. Natl. Acad. Sci. USA, 91: 4096-4100

15   (1994); Y. N. Wong et al., Biopharm. & Drug Dispos. 15:535-
544 (1994); M.L. West and D. P. Fairlie, Trends Pharmacol.
Sci. 16:67-75 (1995); and S. Thaisrivongs, "HIV Protease
Inhibitors", Ann. Reports Med. Chem., Vol. 29, Chap. 14, pp.
133-144 (1994) (Academic Press, J. Bristol, Ed.), each of

20   which is incorporated herein by reference in their entirety.
        Without further elaboration, it is believed that one
skilled in the art can, using the preceding description,
utilize the present invention to its fullest extent. The
following preferred specific embodiments are not intended to

25   provide an exhaustive description of all possible compound
combinations but merely to provide examples of drug
combinations that are anticipated to be effective. Similar
testing of these and other protease inhibitors using
resistant viral isolates, not limited to those listed below,

30   can help identify suitable drug combinations. Therefore,
the following preferred specific embodiments are to be
construed as merely illustrative, and not limitative of the
remainder of the disclosure in any way whatsoever.

Example 1
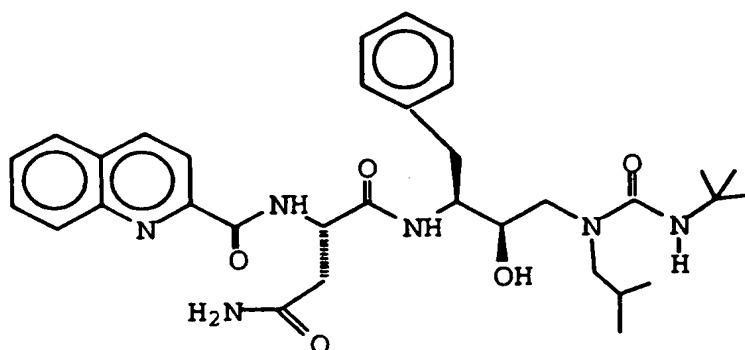
[1S-[1R*(R*),2S*]]-N^1-[3-[[[(1,1-
dimethylethyl)amino]carbonyl]])2-methylpropyl)amino]-2-
hydroxy-1-(phenylmethyl)propyl]-2-[(2-
quinolinylcarbonyl)amino]-butanediamide can be prepared
according to the methods disclosed in co-owned and co-
pending US Patent Applications Serial Nos. 08/152,934 (filed
November 15, 1993) and 08/156,498 (filed November 23, 1993),
both incorporated herein by reference in their entirety.

## Example 2
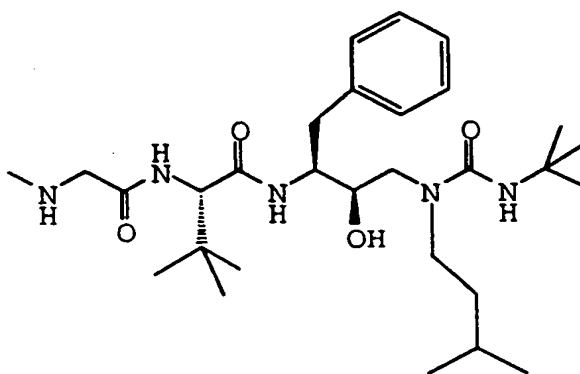
(2R,3S)-3-(N-methylaminoacetyl-L-tert-butylglycinyl)amino-1-
(N-isoamyl-N-(tert-butylcarbamoyl))amino-4-phenyl-2-butanol
can be prepared according to the methods disclosed in co-

owned and co-pending US Patent Applications Serial Nos.
08/109,787 (filed August 20, 1993), Attorney docket No.
2766/1 co-filed with the present application, and 08/156,498
(filed November 23, 1993, all three incorporated herein by
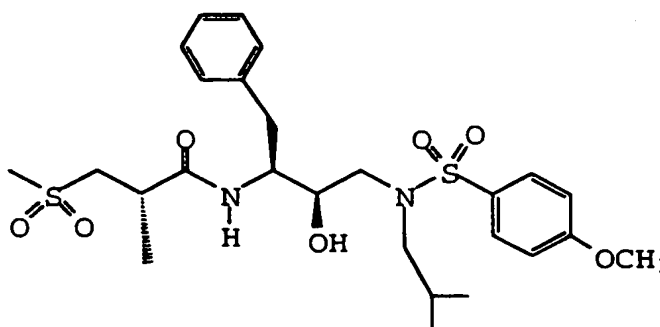5   reference in their entirety.

Example 3
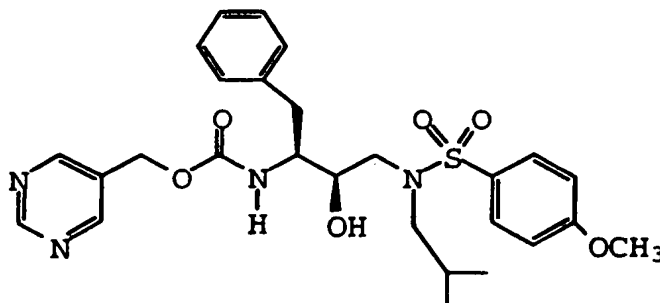
10

[2R-hydroxy-3-[[(4-methoxyphenyl)sulfonyl](2-
methylpropyl)amino]-1S-(phenylmethyl)propyl]carbamic acid 5-
pyrimidylmethyl ester can be prepared according to the
methods disclosed in co-owned and co-pending US Patent
15 Applications Serial Nos. 08/110,911 (filed August 24, 1993)
and 08/156,498 (filed November 23, 1993), both incorporated
herein by reference in their entirety.

Example 4

20

[1S-[1R*(R*),2S*]]-N-[2-hydroxy-3-[N^1-(2-methylpropyl)-N^1-
(4-methoxyphenylsulfonyl)amino]-1-(phenylmethyl)propyl]-2-
methyl-3-(methylsulfonyl)propanamide can be prepared
according to the methods disclosed in co-owned and co-

5   pending US Patent Applications Serial Nos. 08/110,913 (filed
August 24, 1993) and 08/156,498 (filed November 23, 1993),
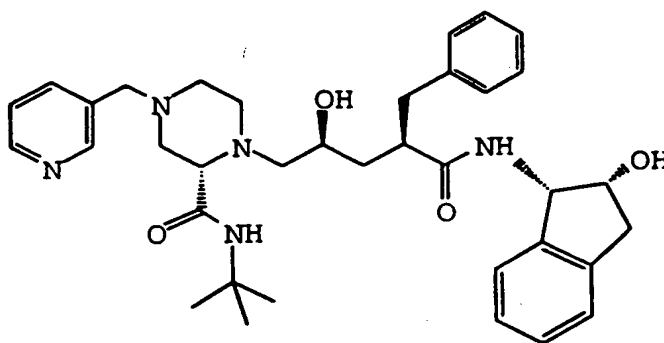both incorporated herein by reference in their entirety.

Example 5

10

N-(2(R)-Hydroxy-1(S)-indanyl)-2(R)-phenylmethyl-4(S)-
hydroxy-5-(1-(4-(3-pyridylmethyl)-2(S)-N'-(t-

15   butylcarboxamido)-piperazinyl))-pentaneamide (L-735,524) can
be prepared according to the methods disclosed in US Patent
Application Serial No. 07/883,825 (filed May 15, 1992), WO
93/09096, Tet. Lett. 35:673-676 (1994) and Proc. Natl. Acad.
Sci. USA, 91:4096-4100 (1994), each of which is incorporated

20   herein by reference in its entirety.

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Example 6
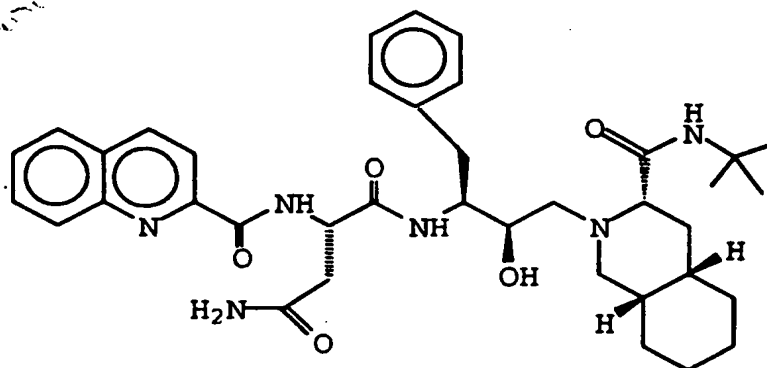
5   N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-
     quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aR,8aS)-
     isoquinoline-3(S)-carboxamide (Ro 31-8959) can be prepared
     according to the methods disclosed in US Patent 5,157,041,
     incorporated herein by reference in its entirety.

10

Example 7

15   [1S-[1R*(R*),2S*]]-N^1-[3-[[[(1,1-dimethylethyl)
     amino]carbonyl](3-methylbutyl)amino]-2-hydroxy-1-
     (phenylmethyl)propyl]-2-[(2-quinolinylcarbonyl)amino]-
     butanediamide can be prepared according to the methods
     disclosed in co-owned and co-pending US Patent Applications
20   Serial Nos. 08/152,934 (filed November 15, 1993) and

12

08/156,498 (filed November 23, 1993), both incorporated
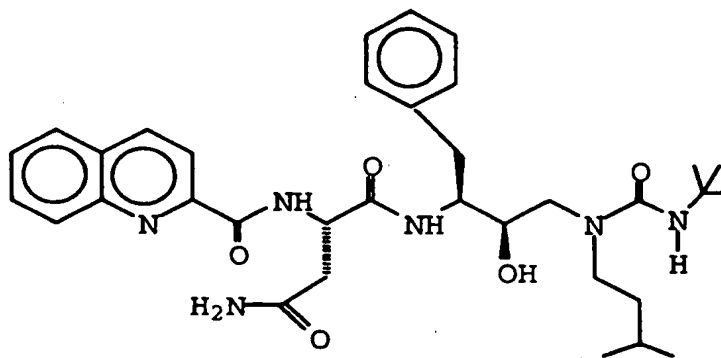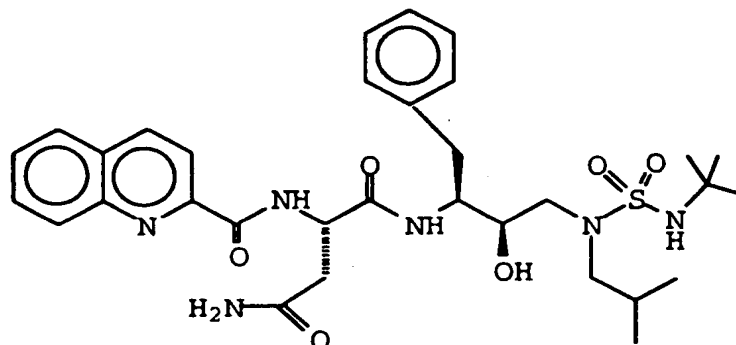herein by reference in their entirety.

<u>Example 8</u>

5

N-[3-[N^2-[N^1-(1,1-dimethylethyl)aminosulfonyl]-N^2-(2-
methylpropyl)amino]-2R-hydroxy-1S-(phenylmethyl)propyl]-2S-
10   [(2-quinolinylcarbonyl)amino]butanediamide can be prepared
according to the methods disclosed in co-owned and co-
pending PCT Patent Application No. PCT/US93/10552 (filed
October 29, 1993) and US Patent Application Serial No.
08/156,498 (filed November 23, 1993), both incorporated
15   herein by reference in their entirety.

<u>Example 9</u>
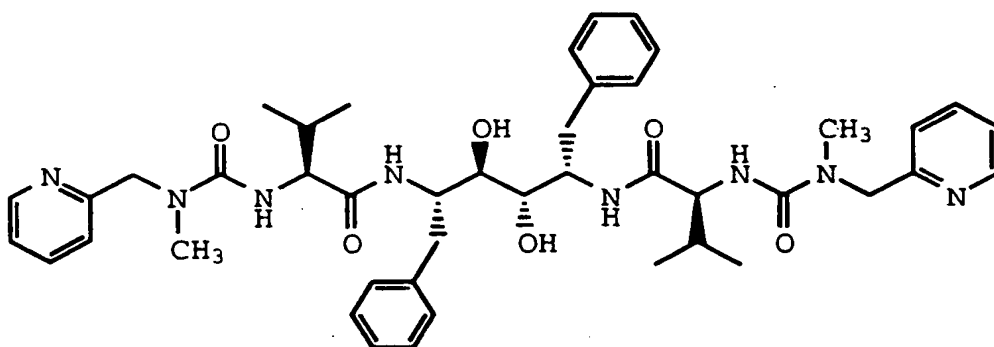
20

(2S,3R,4S,5S)-2,5-Bis-[N-[N-[[N-methyl-N-(2-
pyridinylmethyl)amino]carbonyl]valinyl]amino]-3,4-dihydroxy-
1,6-diphenylhexane (A-77003) can be prepared according to
the methods disclosed in J. Med. Chem. 36:320-330 (1993),
5   which is incorporated herein by reference in its entirety.

Example 10
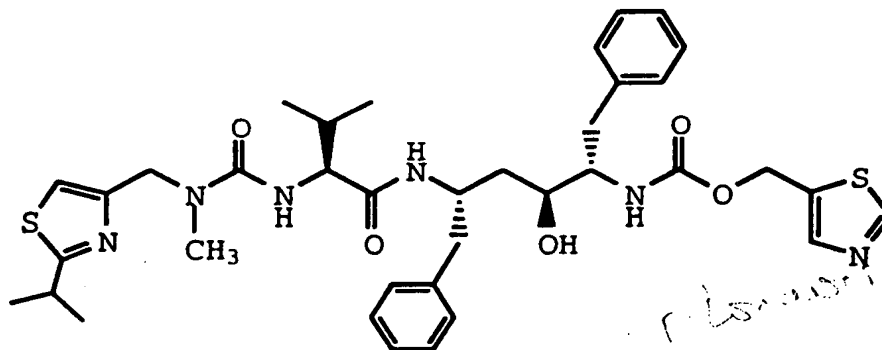
10

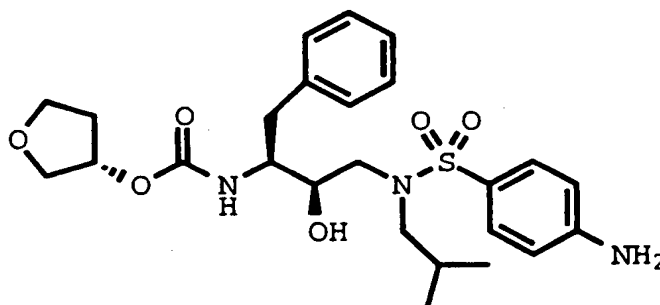(2S,3S,5S)-5-[N-[N-[N-methyl-N-[(2-isopropyl-4-
thiazolyl)methyl]amino]carbonyl]valinyl]amino]-2-[N-[(5-
thiazolyl)methoxycarbonyl]amino]-3-hydroxy-1,6-
diphenylhexane (A-84538, ABT-538) can be prepared according
15   to the methods disclosed in PCT Patent Application Serial
No. WO 94/14436 (filed December 16, 1993), which is
incorporated herein by reference in its entirety.

Example 11

20

[2R-hydroxy-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]carbamic acid 3S-tetrahydrofuranyl ester (VX-478) can be prepared according to the methods disclosed in PCT Patent
5   Applications Serial No. WO 94/05639 (filed September 7, 1993), incorporated herein by reference in its entirety.

Example 12
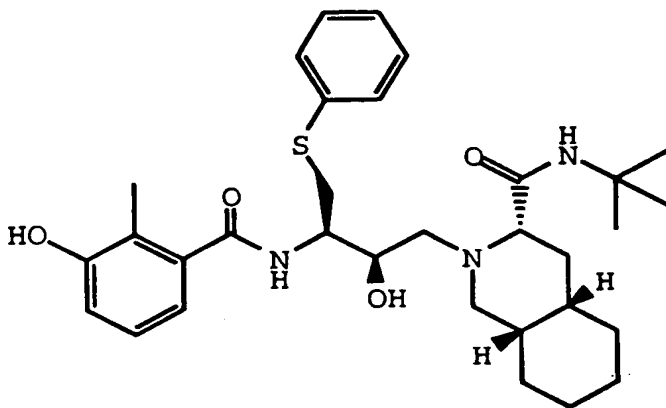
10

N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-(phenylthio)-3(S)-[[N-[(2-methyl-3-hydroxyphenyl)carbonyl]amino]butyl]-(4aR,8aS)-isoquinoline-3(S)-carboxamide (AG-1343, AG-1350)
15   can be prepared according to the methods disclosed in Bioorg. & Med. Chem. Let. 5:715-720, 5:721-726 and 5:727-732 (1995), each of which is incorporated herein by reference in their entirety. In particular, the HOBT active ester of 3-hydroxy-2-methylbenzoic acid (Bioorg. & Med. Chem. Let.
20   5:727-732 (1995)) can be coupled to N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-(phenylthio)-3(S)-aminobutyl]-(4aR,8aS)-isoquinoline-3(S)-carboxamide (Bioorg. & Med. Chem. Let. 5:715-720 (1995)).
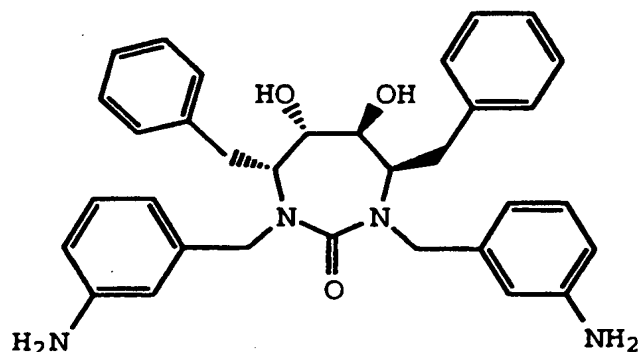
15

Example 13

5   [4R-(4α,5α,6ß,7ß)]-1,3-bis[(3-aminophenyl)methyl]hexahydro-
     5,6-dihydroxy-4,7-bis(phenylmethyl)-2H-1,3-diazepin-2-one
     (DMP-450, XM-412) can be prepared according to the methods
     disclosed in PCT Patent Application WO 93/07128,
     incorporated herein by reference in its entirety. In
10   particular, 3-nitrophenylmethyl halide, such as 3-
     nitrophenylmethylchloride or bromide, is reacted with the
     hydroxy protected derivative of [4R-(4α5α,6ß,7ß)]-
     hexahydro-5,6-dihydroxy-4,7-bis(phenylmethyl)-2H-1,3-
     diazepin-2-one followed by deprotection of the hydroxy
15   groups (see WO 93/07128) and reduction of the nitro groups
     to the amino groups. Such reductions can be accomplished
     using standard procedures well known to those skilled in the
     art.

20   Example 14
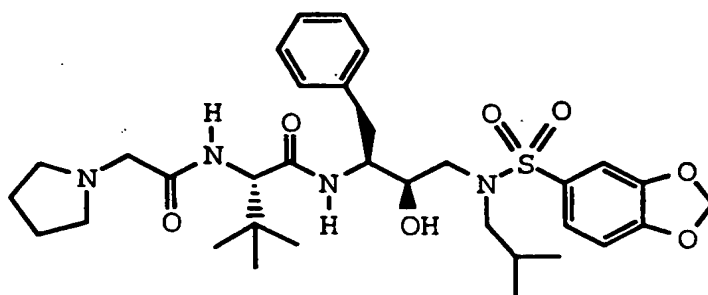
Preparation of N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)
sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl) propyl]-
2S-[[(pyrrolidin-1-yl)acetyl]amino]-3,3-dimethylbutanamide

Part A: Preparation of 1,3-benzodioxole-5-sulfonyl chloride

To a solution of 4.25 g of anhydrous N,N-
dimethylformamide at 0°C under nitrogen was added 7.84g of
sulfuryl chloride, whereupon a solid formed. After stirring
for 15 minutes, 6.45 g of 1,3-benzodioxole was added, and
the mixture heated at 100°C for 2 hours. The reaction was
cooled, poured into ice water, extracted with methylene
chloride, dried over magnesium sulfate, filtered and
concentrated to give 7.32 g of crude material as a black
oil. This was chromatographed on silica gel using 20%
methylene chloride/hexane to afford 1.9 g of (1,3-
benzodioxol-5-yl)sulfonyl chloride.

Alternaively, to a 22 liter round bottom flask fitted
with a mechanical stirrer, a cooling condenser, a heating
mantle and a pressure equalizing dropping funnel was added
sulfur trioxide DMF complex (2778g, 18.1 moles).
Dichloroethane (4 liters) was then added and stirring
initiated. 1,3-Benzodioxole (1905g, 15.6 moles) as then
added through the dropping funnel over a five minute period.
The temperature was then raised to 75°C and held for 22
hours (NMR indicated that the reaction was done after 9
hours.) The reaction was cooled to 26° and oxalyl chloride
(2290g, 18.1 moles) was added at a rate so as to maintain
the temperature below 40°C (1.5 hours). The mixture was
heated to 67°C for 5 hours followed by cooling to 16°C with
an ice bath. The reaction was quenched with water (5 l) at
a rate which kept the temperature below 20°C. After the
addition of water was complete, the mixture was stirred for

10 minutes. The layers were separated and the organic layer
was washed again twice with water (5l). The organic layer
was dried with magnesium sulfate (500g) and filtered to
remove the drying agent. The solvent was removed under
5   vacuum at 50°C. The resulting warm liquid was allowed to
cool at which time a solid began to form. After one hour,
the solid was washed with hexane (400 mL), filtered and
dried to provide the desired sulfonyl chloride (2823g). The
hexane wash was concentrated and the resulting solid washed
10   with 400 mL hexane to provide additional sulfonyl chloride
(464g). The total yield was 3287g (95.5% based upon 1,3-
benzodioxole).

Part B: Preparation of 2S-[Bis(phenylmethyl)amino]-3-
15   phenylpropanol

METHOD 1:   2S-[Bis(phenylmethyl)amino]-3-phenylpropanol from
the DIBAL Reduction of N,N-bis(phenylmethyl)-L-Phenylalanine
phenylmethyl ester
20

Step 1:   A solution of L-phenylalanine (50.0 g, 0.302 mol),
sodium hydroxide (24.2 g, 0.605 mol) and potassium carbonate
(83.6 g, 0.605 mol) in water (500 mL) was heated to 97°C.
Benzyl bromide (108.5 mL, 0.605 mol) was then slowly added
25   (addition time - 25 min). The mixture was stirred at 97°C
for 30 minutes under a nitrogen atmosphere. The solution
was cooled to room temperature and extracted with toluene (2
x 250 mL). The combined organic layers were washed with
water and brine, dried over magnesium sulfate, filtered and
30   concentrated to an oil. N,N-bis(phenylmethyl)-L-
phenylalanine phenylmethyl ester can be purified by column
chromatography (silica gel, 15% ethyl acetate/hexane).
Usually the product is pure enough to be used directly in
the next step without further purification. EIMS: m/z 434
35   (M-1).

Step 2:   The benzylated phenylalanine phenylmethyl ester
(0.302 mol) from the previous reaction was dissolved in
toluene (750 mL) and cooled to -55°C. A 1.5 M solution of
5   DIBAL in toluene (443.9 mL, 0.666 mol) was added at a rate
to maintain the temperature between -55 to -50°C (addition
time - 1 hr). The mixture was stirred for 20 minutes under
a nitrogen atmosphere and then quenched at -55°C by the slow
addition of methanol (37 ml). The cold solution was then
10   poured into cold (5°C) 1.5 N HCl solution (1.8 L). The
precipitated solid (approx. 138 g) was filtered off and
washed with toluene. The solid material was suspended in a
mixture of toluene (400 mL) and water (100 ml). The mixture
was cooled to 5°C and treated with 2.5 N NaOH (186 mL) and
15   then stirred at room temperature until solid dissolved. The
toluene layer was separated from the aqueous phase and
washed with water and brine, dried over magnesium sulfate,
filtered and concentrated to a volume of 75 mL (89 g).
Ethyl acetate (25 mL) and hexane (25 mL) were added to the
20   residue upon which the desired alcohol product began to
crystallize. After 30 min, an additional 50 mL hexane were
added to promote further crystallization. The solid was
filtered off and washed with 50 mL hexane to give 34.9 g of
first crop product. A second crop of product (5.6 g) was
25   isolated by refiltering the mother liquor. The two crops
were combined and recrystallized from ethyl acetate (20 mL)
and hexane (30 mL) to give 40 g of 2S-[Bis(phenyl-
methyl)amino]-3-phenylpropanol, 40% yield from
L-phenylalanine. Anal. Calcd. for $C_{23}H_{25}ON$: C, 83.34; H,
30   7.60; N, 4.23. Found: C, 83.43; H, 7.59; N, 4.22.

METHOD 2:   Preparation of ßS-2-[Bis(phenylmethyl)amino]
benzene-propanol from the N,N-Dibenzylation of
L-Phenylalaninol

35

19

L-phenylalaninol (176.6 g, 1.168 mol) was added to a
stirred solution of potassium carbonate (484.6 g, 3.506 mol)
in 710 mL of water. The mixture was heated to 65°C under a
nitrogen atmosphere. A solution of benzyl bromide (400 g,
5   2.339 mol) in 3A ethanol (305 mL) was added at a rate that
maintained the temperature between 60-68°C. The biphasic
solution was stirred at 65°C for 55 min and then allowed to
cool to 10°C with vigorous stirring. The oily product
solidified into small granules. The product was diluted with
10   2.0 L of tap water and stirred for 5 minutes to dissolve the
inorganic by products. The product was isolated by filtration
under reduced pressure and washed with water until the pH is
7. The crude product obtained was recrystallized from 1.1 L
of ethyl acetate/heptane (1:10). The product was isolated by
15   filtration (at -8°C), washed with 1.6 L of cold (-10°C) ethyl
acetate/heptane (1:10) and air-dried to give 339 g (88% yield)
of 2S-[Bis(phenylmethyl)amino]-3-phenylpropanol, Mp = 71.5-
73.0°C.

20   Part C: Preparation of 2S-[Bis(phenylmethyl)amino]-3-
       phenylpropanaldehyde

       METHOD 1:   2S-[Bis(phenylmethyl)amino]-3-phenylpropanol (200
       g, 0.604 mol) was dissolved in triethylamine (300 mL, 2.15
25   mol). The mixture was cooled to 12°C and a solution of
       sulfur trioxide/pyridine complex (380 g, 2.39 mol) in DMSO
       (1.6 L) was added at a rate to maintain the temperature
       between 8-17°C. The solution was stirred at ambient
       temperature under a nitrogen atmosphere for 1.5 hour. The
30   reaction mixture was cooled with ice water and quenched with
       1.6 L of cold water (10-15°C) over 45 minutes. The
       resultant solution was extracted with ethyl acetate (2.0 L),
       washed with 5% citric acid (2.0 L) and brine (2.2 L), dried
       over MgSO4 (280 g) and filtered. The solvent was removed in
35   vacuo and then dried under vacuum to give 198.8 g of 2S-

[Bis-(phenylmethyl)amino]-3-phenylpropanaldehyde as a pale
yellow oil (99.9%). The crude product obtained was pure
enough to be used directly in the next step without
purification.

METHOD 2: A solution of oxalyl chloride (8.4 ml, 0.096 mol)
in dichloromethane (240 ml) was cooled to -74°C. A solution
of DMSO (12.0 ml, 0.155 mol) in dichloromethane (50 ml) was
then slowly added at a rate to maintain the temperature at
-74°C (addition time ~1.25 hr). The mixture was stirred for
5 min. followed by addition of a solution of 2S-
[bis(phenylmethyl)amino]-3-phenylpropanol (0.074 mol) in 100
ml of dichloromethane (addition time ~20 min., temp. -75°C
to -68°C). The solution was stirred at -78°C for 35 minutes
under a nitrogen atmosphere. Triethylamine (41.2 ml, 0.295
mol) was then added over 10 min. (temp. -78° to -68°C) upon
which the ammonium salt precipitated. The cold mixture was
stirred for 30 min. and then water (225 ml) was added. The
dichloromethane layer was separated from the aqueous phase
and washed with water, brine, dried over magnesium sulfate,
filtered and concentrated. The residue was diluted with
ethyl acetate and hexane and then filtered to further remove
the ammonium salt. The filtrate was concentrated to give
2S-[bis(phenylmethyl)amino]-3-phenylpropanaldehyde. The
aldehyde was carried on to the next step without
purification.

METHOD 3: To a mixture of 1.0 g (3.0 mmoles) of 2S-
[bis(phenylmethyl)amino]-3-phenylpropanol 0.531 g (4.53
mmoles) of N-methylmorpholine, 2.27 g of molecular sieves
(4A) and 9.1 mL of acetonitrile was added 53 mg (0.15
mmoles) of tetrapropylammonium perruthenate (TPAP). The
mixture was stirred for 40 minutes at room temperature and
concentrated under reduced pressure. The residue was
suspended in 15 mL of ethyl acetate, filtered through a pad

of silica gel. The filtrate was concentrated under reduced
pressure to give a product containing approximately 50% of
2S-[bis(phenylmethyl)amino]-3-phenylpropanaldehyde as a pale
yellow oil.

5

METHOD 4: To a solution of 1.0 g (3.02 mmoles) of 2S-
[bis(phenylmethyl)amino]-3-phenylpropanol in 9.0 mL of
toluene was added 4.69 mg (0.03 mmoles) of 2,2,6,6-
tetramethyl-1-piperidinyloxy, free radical (TEMPO),
10   0.32g(3.11 mmoles) of sodium bromide, 9.0 mL of ethyl
acetate and 1.5 mL of water. The mixture was cooled to 0°C
and an aqueous solution of 2.87 mL of 5% household bleach
containing 0.735 g (8.75 mmoles) of sodium bicarbonate and
8.53 mL of water was added slowly over 25 minutes. The
15   mixture was stirred at 0°C for 60 minutes. Two more
additions (1.44 mL each) of bleach was added followed by
stirring for 10 minutes. The aqueous layer was extracted
twice with 20 mL of ethyl acetate. The combined organic
layer was washed with 4.0 mL of a solution containing 25 mg
20   of potassium iodide and water (4.0 mL), 20 mL of 10% aqueous
sodium thiosulfate solution and then brine solution. The
organic solution was dried over magnesium sulfate, filtered
and concentrated under reduced pressure to give 1.34g of
crude oil containing a small amount of the desired product
25   aldehyde, 2S-[bis(phenylmethyl)amino]-3-phenyl
propanaldehyde.

Part D: Preparation of N,N-dibenzyl-3(S)-amino-1,2-(S)-
epoxy-4-phenylbutane

30

METHOD 1:   A solution of 2S-[Bis(phenylmethyl)amino]-3-
phenylpropanaldehyde (191.7 g, 0.58 mol) and
chloroiodomethane (56.4 mL, 0.77 mol) in tetrahydrofuran
(1.8 L) was cooled to -30 to -35°C in a stainless steel
35   reactor under a nitrogen atmosphere. A solution of n-butyl

lithium in hexane (1.6 M, 365 mL, 0.58 mol) was then added
at a rate that maintained the temperature below -25°C.
After addition the mixture was stirred at -30 to -35°C for
10 minutes.   More additions of reagents were carried out in
5   the following manner: (1) additional chloroiodomethane (17
mL) was added, followed by n-butyl lithium (110 mL) at <
-25°C.   After addition the mixture was stirred at -30 to
-35°C for 10 minutes.   This was repeated once.   (2)
Additional chloroiodomethane (8.5 mL, 0.11 mol) was added,
10   followed by n-butyl lithium (55 mL, 0.088 mol) at <-25°C.
After addition the mixture was stirred at -30 to -35°C for
10 minutes.   This was repeated 5 times.   (3) Additional
chloroiodomethane (8.5 mL, 0.11 mol) was added, followed by
n-butyl lithium (37 mL, 0.059 mol) at <-25°C.   After
15   addition the mixture was stirred at -30 to -35°C for 10
minutes.   This was repeated once.   The external cooling was
stopped and the mixture warmed to ambient temp. over 4 to 16
hours when TLC (silica gel, 20% ethyl acetate/hexane)
indicated that the reaction was completed.   The reaction
20   mixture was cooled to 10°C and quenched with 1452 g of 16%
ammonium chloride solution, keeping the temperature below
23°C.   The mixture was stirred for 10 minutes and the
organic and aqueous layers were separated.   The aqueous
phase was extracted with ethyl acetate (2x 500 mL).   The
25   ethyl acetate layer was combined with the tetrahydrofuran
layer.   The combined solution was dried over magnesium
sulfate (220g), filtered and concentrated in vacuo.   The
brown oil residue was dried at 70°C in vacuo (0.8 bar) for 1
hour to give 222.8 g of crude material.   The crude product
30   is usually used directly in the next step without
purification.

METHOD 2:   A solution of the crude aldehyde 0.074 mol and
chloroiodomethane (7.0 ml, 0.096 mol) in tetrahydrofuran
35   (285 ml) was cooled to -78°C, under a nitrogen atmosphere.

A 1.6 M solution of n-butyl lithium in hexane (25 ml, 0.040
mol) was then added at a rate to maintain the temperature at
-75°C. After the first addition, additional
chloroiodomethane (1.6 ml, 0.022 mol) was added again,
5   followed by n-butyl lithium (23 ml, 0.037 mol), keeping the
temperature at -75°C. The mixture was stirred for 15 min.
Each of the reagents, chloroiodomethane (0.70 ml, 0.010 mol)
and n-butyl lithium (5 ml, 0.008 mol) were added 4 more
times over 45 min. at -75°C. The cooling bath was then
10   removed and the solution warmed to 22°C over 1.5 hr. The
mixture was poured into 300 ml of saturated aq. ammonium
chloride solution. The tetrahydrofuran layer was separated.
The aqueous phase was extracted with ethyl acetate (1 x 300
ml). The combined organic layers were washed with brine,
15   dried over magnesium sulfate, filtered and concentrated to
give a brown oil (27.4 g). The product could be used in the
next step without purification.

METHOD 3: A solution of 2S-[Bis(phenylmethyl)amino]-3-
20   phenylpropanaldehyde (178.84 g, 0.54 mol) and
bromochloromethane (46 mL, 0.71 mol) in tetrahydrofuran (1.8
L) was cooled to -30 to -35°C in a stainless steel reactor
under a nitrogen atmosphere. A solution of n-butyl lithium
in hexane (1.6 M, 340 mL, 0.54 mol) was then added at a rate
25   that maintained the temperature below -25°C. After addition
the mixture was stirred at -30 to -35°C for 10 minutes.
More additions of reagents were carried out in the following
manner: (1) additional bromochloromethane (14 mL) was
added, followed by n-butyl lithium (102 mL) at < -25°C.
30   After addition the mixture was stirred at -30 to -35°C for
10 minutes. This was repeated once. (2) Additional
bromochloromethane (7 mL, 0.11 mol) was added, followed by
n-butyl lithium (51 mL, 0.082 mol) at <-25°C. After
addition the mixture was stirred at -30 to -35°C for 10
35   minutes. This was repeated 5 times. (3) Additional

bromochloromethane (7 mL, 0.11 mol) was added, followed by
n-butyl lithium (51 mL, 0.082 mol) at <-25°C. After
addition the mixture was stirred at -30 to -35°C for 10
minutes. This was repeated once. The external cooling was
5   stopped and the mixture warmed to ambient temp. over 4 to 16
hours when TLC (silica gel, 20% ethyl acetate/hexane)
indicated that the reaction was completed. The reaction
mixture was cooled to 10°C and quenched with 1452 g of 16%
ammonium chloride solution, keeping the temperature below
10   23°C. The mixture was stirred for 10 minutes and the
organic and aqueous layers were separated. The aqueous
phase was extracted with ethyl acetate (2x 500 mL). The
ethyl acetate layer was combined with the tetrahydrofuran
layer. The combined solution was dried over magnesium
15   sulfate (220 g), filtered and concentrated on a rotary
evaporator at 65°C. The brown oil residue was dried at 70°C
in vacuo (0.8 bar) for 1 hour to give 222.8 g of crude
material.

20   Part E: Preparation of N-[3(S)-[N,N-bis(phenylmethyl)
amino]-2(R)-hydroxy-4-phenylbutyl]-N-isobutylamine•oxalic
acid salt

Step 1: To a solution of crude N,N-dibenzyl-3(S)-amino-
25   1,2(S)-epoxy-4-phenylbutane (388.5 g, 1.13 mol) in
isopropanol (2.7 L) (or ethyl acetate) was added
isobutylamine (1.7 kgm, 23.1 mol) over 2 min. The
temperature increased from 25°C and to 30°C. The solution
was heated to 82°C and stirred at this temperature for 1.5
30   hours. The warm solution was concentrated in vacuo. The
brown oil residue was dried in vacuo (0.8 mm Hg) for 16 h to
give 450 g of product as a crude oil.

Step 2: To a solution of oxalic acid (8.08g, 89.72 mmol) in
35   methanol (76 mL) was added a solution of crude 3(S)-[N,N-

dried over anhydrous magnesium sulfate. After filtering and
washing the magnesium sulfate with ethyl acetate (200 mL),
the solvent was removed in vacuo to yield the desired
sulfonamide as an viscous yellow foamy oil (440.2g 105%
5   yield). HPLC/MS (electrospray) (m/z 601 [M+H]+).

     Alternatively, N-[3(S)-[N,N-bis(phenylmethyl)amino]-
2(R)-hydroxy-4-phenylbutyl]-N-isobutylamine•oxalic acid salt
(2800g, 5.53 moles) and THF (4L) were added to a 22L round
10   bottom flask fitted with a mechanical stirrer. Potassium
carbonate (1921g, 13.9 moles) was dissolved in water (2.8L)
and added to the THF slurry. The mixture was then stirred
for one hour. 1,3-benzodioxole-5-sulfonyl chloride (1281g,
5.8 moles) was dissolved in THF (1.4L) and added to the
15   reaction mixture over 25 minutes. An additional 200 mL of
THF was used to rinse the addition funnel. The reaction was
allowed to stir for 14 hours and then water (4 L) was added.
This mixture was stirred for 30 minutes and the layers
allowed to separate. The layers was removed and the aqueous
20   layer washed twice with THF (500 mL). The combined THF
layers were dried with magnesium sulfate (500 g) for one
hour. This solution was then filtered to remove the drying
agent and used in subsequent reactions.

25   Part G: Preparation of 1-[N-[(1,3-benzodioxol-5-yl)
sulfonyl]-N-(2-methylpropyl)amino]-3(S)-amino-4-phenyl-2(R)-
butanol•methanesulfonic acid salt

     Crude 1-[N-[(1,3-benzodioxol-5-yl)sulfonyl]-N-(2-
30   methylpropyl)amino]-3(S)-[bis(phenylmethyl)amino]-4-phenyl-
2(R)-butanol (6.2g, 0.010 moles) was dissolved in methanol
(40 mL). Methanesulfonic acid (0.969g, 0.010 moles) and
water (5 mL) were then added to the solution. The mixture
was placed in a 500 mL Parr hydrogenation bottle containing
35   20% Pd(OH)$_2$ on carbon (255 mg, 50% water content). The

bis(phenylmethyl)amino]-1-(2-methylpropyl)amino-4-
phenylbutan-2(R)-ol in ethyl acetate (90 mL) over 15
minutes. The mixture was stirred at room temperature for
about 2 hours. Solid was isolated by filtration, washed

5   with ethyl acetate (2 x 20 mL) and dried in vacuo for about
1 hour to yield 21.86g of 97% diastereomerically pure salt.
Mp = 174.99°C; Microanalysis: Calc.: C 71.05%, H 7.50%, N
5.53%; Found: C 71.71%, H 7.75%, N 5.39%.

10   Alternatively, crude 3(S)-[N,N-bis(phenylmethyl)
amino]-1-(2-methylpropyl)amino-4-phenylbutan-2(R)-ol (5 g)
was dissolved in methyl-tert-butylether (MTBE) (10 mL) and
oxalic acid (1 g) in methanol (4 mL) was added. The mixture
was stirred for about 2 hours. The resulting solid was

15   filtered, washed with cold MTBE and dried to yield 2.1 g of
white solid of about 98.9% diastereomerically pure (based on
HPLC peak areas).

Part F: Preparation of 1-[N-[(1,3-benzodioxol-5-yl)
20   sulfonyl]-N-(2-methylpropyl)amino]-3(S)-[N,N-
bis(phenylmethyl)amino]-4-phenyl-2(R)-butanol

       To N-[3(S)-[N,N-bis(phenylmethyl)amino]-2(R)-hydroxy-4-
phenylbutyl]-N-isobutylamine•oxalic acid salt (354.7 g, 0.7
25   mole) in 1,4-dioxane (2000 mL) was added a solution of
potassium carbonate (241.9 g, 1.75 moles) in water (250 mL).
The mixture was stirred for 2 hours at room temperature
followed by addition of 1,3-benzodioxole-5-sulfonyl chloride
(162.2 g, 0.735 mole) in 1,4-dioxane (250 mL) over 15
30   minutes. The reaction mixture was stirred at room
temperature for 18 hours. Ethyl acetate (1000 mL) and water
(500 mL) were added and stirring continued for another 1
hour. The aqueous layer was separated and further extracted
with ethyl acetate (200 mL). The combined ethyl acetate
35   layers were washed with 25% brine solution (500 mL) and

bottle was placed in the hydrogenator and purged 5 times
with nitrogen and 5 times with hydrogen. The reaction was
allowed to proceed at 35°C with 63 PSI hydrogen pressure for
18 hours. Additional catalyst (125 mg) was added and, after
5   purging, the hydrogenation continued for and additional 20
hours. The mixture was filtered through celite which was
washed with methanol (2 X 10 mL). Approximately one third
of the methanol was removed under reduced pressure. The
remaining methanol was removed by aziotropic distillation
10   with toluene at 80 torr. Toluene was added in 15, 10, 10
and 10 mL portions. The product crystallized from the
mixture and was filtered and washed twice with 10 mL
portions of toluene. The solid was dried at room
temperature at 1 torr for 6 hours to yield the amine salt
15   (4.5 g, 84%): m/z 421 [M+H]+.

Alternaively, to a THF solution of crude 1-[N-[(1,3-
benzodioxol-5-yl)sulfonyl]-N-(2-methylpropyl)amino]-3(S)-
[bis(phenylmethyl)amino]-4-phenyl-2(R)-butanol was added
20   water (500 mL) followed by methanesulfonic acid (531g, 5.5
moles). The solution was stirred to insure complete mixing
and added to a 5 gallon autoclave. Pearlman's catalyst
(200g of 20% Pd(OH)$_2$ on C/ 50% water) was added to the
autoclave with the aid of THF (500 mL). The reactor was
25   purged four times with nitrogen and four times with
hydrogen. The reactor was charged with 60 psig of hydrogen
and stirring at 450 rpm started. After 16 hours, HPLC
analysis indicated that a small amount of the mono-benzyl
intermediate was still present. Additional catalyst (50g)
30   was added and the reaction was allowed to run overnight.
The solution was then filtered through celite (500g) to
remove the catalyst and concentrated under vacuum in five
portions. To each portion, toluene (500 mL) was added and
removed under vacuum to azeotropically removed residual
35   water. The resulting solid was divided into three portions

and each washed with methyl *t*-butyl ether (2 L) and
filtered. The residual solvent was removed at room
temperature in a vacuum oven at less than 1 torr to yield
the 2714g of the expected salt.

5

Part H: Preparation of N-[2R-hydroxy-3-[[(1,3-benzodioxol-
5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)
propyl]-2S-[(phenylmethoxycarbonyl)amino]-3,3-
dimethylbutanamide

10

To a solution of 118.8 g (0.776 mol) of N-
hydroxybenzotriazole and 137.1 g (0.52 mol) of N-
carbobenzyloxycarbonyl-L-tert-leucine in 750 mL of anhydrous
DMF at 0°C under a nitrogen atmosphere, was added 109.1 g
15   (0.57 mol) of EDC. After stirring at 0°C for 2 hours, a
solution of 273 g (0.53 mol) of 2R-hydroxy-3-[[(1,3-
benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-
(phenylmethyl)propylamine methanesulfonate, previously
neutralized with 228 mL (210g, 2.08 mol) of 4-
20   methylmorpholine, in 250 mL of anhydrous DMF was added.
After stirring at 0°C for 30 minutes, the mixture stirred at
room temperature for 18 hours. The solvents were removed
under reduced pressure at 45°C, 1.5 L of ethyl acetate
added, washed with 5% citric acid, saturated sodium
25   bicabonate, brine, dried over anhydrous magnesium sulfate,
filtered and concentrated to afford 400g of crude material.
This was chromatographed in 3 batches on a Prep 2000
Chromatogram on silica gel using 20%-50% ethyl
acetate/hexane as eluent to yield 320g of purified material,
30   m/e=674 (M+Li), 98% by HPLC.

Part I: Preparation of N-[2R-hydroxy-3-[[(1,3-benzodioxol-
5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)
propyl]-2S-amino-3,3-dimethylbutanamide

35

A solution of 312g of N-[2R-hydroxy-3-[[(1,3-
benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-
(phenylmethyl) propyl]-2S-[(phenylmethoxycarbonyl)amino]-
3,3-dimethylbutanamide in 1L of tetrahydrofuran was
5   hydrogenated in the presence of 100g of 4% palladium-on-
carbon catalyst under 60 psig of hydrogen for 6 hours at
room temperature. The catalyst was removed by filtration
and the solvents removed under reduced pressure to afford
240g of the desired compound.
10

Part J: Preparation of N-[2R-hydroxy-3-[[(1,3-benzodioxol-
5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)
propyl]-2S-[(chloroacetyl)amino]-3,3-dimethylbutanamide

15   To a solution of 234.3 g (0.439 mol) of N-[2R-hydroxy-
3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-
1S-(phenylmethyl) propyl]-2S-amino-3,3-dimethylbutanamide in
1L of methylene chloride, was added 80 mL (59.5 g, 0.46 mol)
of diisopropylethylamine, followed by the slow addition at
20   room temperature of 78.8 g (0.46 mol) of chloroacetic
anhydride while maintaining the temperature below 35°C.
After stirring for an additional 1 hour, analysis by HPLC
indicated a small amount of starting material was still
present, and 1.5 g of chloroacetic anhydride was added.
25   After 10 minutes, the solvents were removed under reduced
pressure, 1 L ethyl acetate added, washed with 5% citric
acid, saturated sodium bicarbonate, brine, dried over
anhydrous magnesium sulfate, filtered and concentrated to
yield 314 g of crude material. This was chromatographeed in
30   3 portions on a Prep 2000 Chromatogram on silica gel using
20-50% ethyl acetate/hexane to afford 165 g of the desired
compound, m/e=616 (M+Li), 98% by HPLC.

Part K: Preparation of N-[2R-hydroxy-3-[[(1,3-benzodioxol-
35   5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)

propyl]-2S-[[(pyrrolidin-1-yl)acetyl]amino]-3,3-
dimethylbutanamide

     To 164.2 g (0.27 mol) of N-[2R-hydroxy-3-[[(1,3-
5   benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-
   (phenylmethyl) propyl]-2S-[(chloroacetyl)amino]-3,3-
dimethylbutanamide was added 500 mL of tetrahydrofuran, the
solvent removed under reduced pressure to remove any ethyl
acetate, and then 350 mL of tetrahydrofuran was added. To
10   this solution at 10°C was added 130 mL (1.56 mol) of
pyrrolidine. After 1 hour, the solvents were removed under
reduced pressure, 1 L ethyl acetate added, washed with
saturated sodium bicarbonate, brine, dried over anhydrous
magnesium sulfate, filtered and concentrated to afford 185 g
15   of crude material, which was assayed by HPLC to be 98.9%
purity. This was split into 3 portions and chromatographed
on a Prep 2000 Chromatogram using first 50% ethyl
acetate/hexane, followed by 5% methanol/ethyl acetate to
afford 160g of purified material (99% by HPLC). This was
20   then recrystallized from 460 mL of diethyl ether and 70 mL
of hexane to afford 121g of the desired product (>99% by
HPLC), m/e=651(M+Li), mp=112-114°C.

Example 15
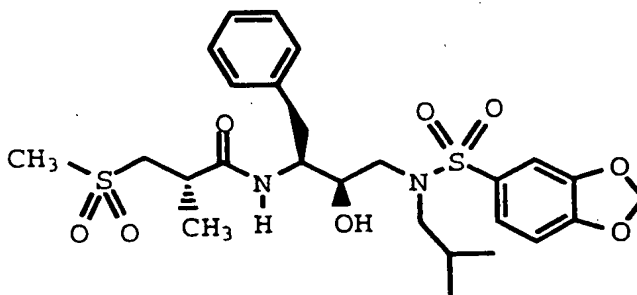
25

<u>Preparation of N-[2R-hydroxy-3-[(2-methylpropyl)[(1,3-benzodioxol-5-yl)sulfonyl]amino]-1S-(phenylmethyl) propyl]-2S-methyl-3-(methylsulfonyl)propanamid</u>e

5   Part A: Preparation of 2(S)-methyl-3-(methylsulfonyl) propionic Acid

Step 1:   To a solution of 200 g (1.23 mol) of D-(-)-3-acetyl-b-mercaptoisobutyric acid in 1.0 L of methanol, was
10   added 161.0 g (2.47 mol) of potassium hydroxide dissolved in 500 mL of methanol while maintaining the temperature below 10°C while cooling with an ice bath. After stirring an additional 20 minutes, 117 mL (156 g, 1.23 mol) of dimethyl sulfate was added while maintaining the temperature below
15   20°C. The ice bath was removed and the mixture stirred for an additional 60 minutes. The salts were removed by filtration, the solvents removed under reduced pressure and ethyl acetate added. After separating the aqueous layer, it was acidified with concentrated hydrochloric acid, extracted
20   with ethyl acetate, dried over anhydrous magnesium sulfate, filtered and concentrated to afford 164 g (99%) of the desired 2S-methyl-3-(methylthio)propionic acid, m/e = 133 (M-H).

25   Step 2:   To a solution of 10.0 g (74.6 mmol) of 2S-methyl-3-(methylthio)propionic acid in 150 mL of acetone and 30 mL of water, cooled to 18°C in an ice bath, was added 161.8 g (263 mmol) of Oxone. After approximately half of material had been added, the temperature rose to 24°C, the addition was
30   stopped, temperature lowered to 18°C, then addition continued. After stirring at 15-20°C for 15 minutes, the bath was removed and the reaction stirred at room temperature for 1 hour. The solids were filtered and washed with acetone, the filtrate concentrated to approximately 40
35   mL and the residue dissolved in 200 mL of ethyl acetate.

The ethyl acetate layer was dried with anhydrous magnesium
sulfate, filtered and concentrated to afford 11.4 g of an
oil. This was dissolved in a minimum of ethyl acetate and
hexane added to cause a precipitate to form. This was

5   collected to afford 6.95 g of the desired product, m/e = 167
(M+H).

Part B: Preparation of N-[2R-hydroxy-3-[(2-methylpropyl)
[(1,3-benzodioxol-5-yl)sulfonyl]amino]-1S-(phenylmethyl)

10 propyl]-2S-methyl-3-(methylsulfonyl)propanamide

To a solution of 5.0g (30 mmol) of 2S-methyl-3-
(methylsulfonyl)propionic acid and 6.90g (45 mmol) N-
hydroxybenzotriazole in 30 mL of anhydrous DMF at 0°C under

15 nitrogen, was added 6.34 g (33 mmol) of EDC. After
approximately 10 minutes, the EDC was all dissolved. After
60 minutes at 0°C, a solution of 15.5 g (30 mmol) of 2R-
hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-
methylpropyl)amino]-1S-(phenylmethyl)propylamine

20 methanesulfonate in 30 mL of anhydrous DMF, previouly
neutralized with 3.4 mL (31.6 mmol) of 4-methylmorpholine,
was added. After 3 hrs at 0°C, the mixture was then stirred
overnight for 17 hrs. The DMF was removed under reduced
pressure, ethyl acetate added, washed with 5% citric acid,

25 saturated sodium bicarbonate, water, brine, dried over
anhydrous magnesium sulfate, filtered and concentrated to
afford 16 g of crude material, which was 88% pure by HPLC.
The product was chromatographed on silica gel using 20%-80%
ethyl acetate/hexane to afford the pure product, which was

30 recrystallized from ethyl acetate/hexane to afford 8.84 g of
pure product, mp 131.8-133.8°C.

Alternatively, to a solution of 35.0g (211 mmol) of
2S-methyl-3-(methylsulfonyl)propionic acid and 48.3g (315

35 mmol) N-hydroxybenzotriazole in 210 mL of anhydrous DMF at

33

0°C under nitrogen, was added 44.4 g (231 mmol) of EDC. After approximately 30 minutes, the EDC was all dissolved. After an additional 60 minutes at 0°C, a solution of 108.8 g (211 mmol) of 2R-hydroxy-3-[[(1,3-benzodioxol-5-

5 yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propylamine methanesulfonate in 350 mL of anhydrous DMF, previouly neutralized with 24 mL (22.3g) of 4-methylmorpholine, was added. After 2 hrs at 0°C, the mixture was then stirred overnight for 18 hrs. The DMF was

10 removed under reduced pressure, 1L of ethyl acetate added, washed with 5% citric acid, saturated sodium bicarbonate, water, brine, dried over anhydrous magnesium sulfate, filtered and concentrated to afford 120.4 g of crude material, which was 90% purity by HPLC. The product was

15 crystallized twice from 750-1000mL of absolute ethanol to afford 82.6 g of the desired product.

<u>Example 16</u>
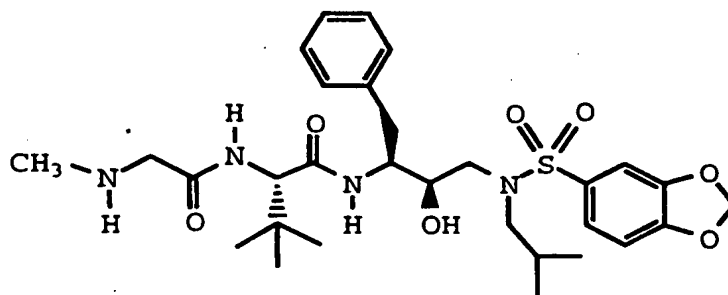
20

<u>Preparation of 2S-[[(N-methylamino)acetyl]amino]-N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-3,3-</u>

25 <u>dimethylbutanamide</u>

To 6.55 g (10.7 mmol) of N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-2S-[(chloroacetyl)amino]-3,3-

dimethylbutanamide was added 25 mL of tetrahydrofuran, the solvent removed under reduced pressure to remove any ethyl acetate, and then 25 mL of tetrahydrofuran was added. To this solution at 10°C was added 19 mL (214 mmol) of 40%

5   aqueous methylamine. After 2 hours, the solvents were removed under reduced pressure, added 1 L ethyl acetate, washed with saturated sodium bicarbonate, brine, dried over anhydrous magnesium sulfate, filtered and concentrated to afford 6.0 g of product (98% purity).

10

Example 17

Retroviral protease inhibitor compounds of the present invention are effective HIV protease inhibitors. The enzyme

15 assay described below can be used in the selection of retroviral protease inhibitors for use in combination therapy. The IC50 (the concentration at which the inhibitor compound reduces enzyme activity by 50%) for such compounds can be calculated using this method.

20   The enzyme method is as follows. The substrate is 2-aminobenzoyl-Ile-Nle-Phe(p-NO2)-Gln-ArgNH2. The positive control is MVT-101 (Miller, M. et al, Science 246, 1149 (1989)). The assay buffer is 20 mM sodium phosphate, pH 6.4, 20% glycerol, 1 mM EDTA, 1 mM DTT and 0.1% CHAPS. The

25 substrate is dissolved in DMSO, then diluted 10 fold in assay buffer. Final substrate concentration in the assay is about 80 μM. HIV protease is diluted in the assay buffer to a final enzyme concentration of about 12.3 nanomolar, based on a molecular weight of 10,780.

30   The final concentration of DMSO is about 14% and the final concentration of glycerol is about 18%. The test compound is dissolved in DMSO and diluted in DMSO to about ten times (10x) the test concentration, then 10 μL of substrate. The increase in fluorescence is monitored at 4

time points (0, 8, 16 and 24 minutes) at ambient
temperature. Each assay is carried out in duplicate wells.

Example 18

5

        The effectiveness of selected HIV protease inhibitor
compounds of the present invention can be determined using
the above-described enzyme assay and the following CD4+ cell
line assay. Antiviral activities of protease inhibitors are
10   expressed as effective concentration 50 (EC_{50}) and/or
effective concentration 90 (EC_{90}) values. These are the
concentrations of inhibitors that were required to inhibit
viral replication by 50% or 90%, respectively.
        The HIV inhibition assay method of acutely infected
15   cells is an automated tetrazolium based colorimetric assay
essentially that reported by Pauwels et al, J. Virol.
Methods 20, 309-321 (1988). Assays are performed in 96-well
tissue culture plates. A CD4+ cell line, such as CEM, MT-2,
MT-4 and the like cell lines, is grown in RPMI-1640 medium
20   (Gibco) supplemented with a 10% fetal calf serum and are
then treated with polybrene (2μg/ml). An 80 μl volume of
medium containing 1×10^4 cells is dispensed into each well
of the tissue culture plate. To each well is added a 100μl
volume of test compound dissolved in tissue culture medium
25   (or medium without test compound as a control) to achieve
the desired final concentration and the cells are incubated
at 37°C for 1 hour. A frozen culture of HIV-1 is diluted in
culture medium to a concentration of 5×10^4 $TCID_{50}$ per ml
($TCID_{50}$ = the dose of virus that infects 50% of cells in
30   tissue culture), and a 20 μL volume of the virus sample
(containing 1000 $TCID_{50}$ of virus) is added to wells
containing test compound and to wells containing only medium
(infected control cells). Several wells receive culture
medium without virus (uninfected control cells). Likewise,
35   the intrinsic toxicity of the test compound is determined by

adding medium without virus to several wells containing test compound. In summary, the tissue culture plates contain the following experiments:

	Cells	Drug	Virus
1.	+	−	−
2.	+	+	−
3.	+	−	+
4.	+	+	+

In experiments 2 and 4 the final concentrations of test compounds are 1, 10, 100 and 500 μg/ml. Either azidothymidine (AZT) or dideoxyinosine (ddI) is included as a positive drug control. Test compounds are dissolved in DMSO and diluted into tissue culture medium so that the final DMSO concentration does not exceed 1.5% in any case. DMSO is added to all control wells at an appropriate concentration.

Following the addition of virus, cells are incubated at 37°C in a humidified, 5% CO_2 atmosphere for 7 days. Test compounds can be added on days 0, 2, and 5 if desired. On day 7, post-infection, the cells in each well are resuspended and a 100μl sample of each cell suspension is removed for assay. A 20μL volume of a 5 mg/ml solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is added to each μL cell suspension, and the cells are incubated for 4 hours at 37° in a 5% CO_2 environment. During this incubation, MTT is metabolically reduced by living cells resulting in the production in the cell of a colored formazan product. To each sample is added 100μl of 10% sodium dodecylsulfate in 0.01 N HCl to lyse the cells, and samples are incubated overnight. The absorbance at 590 nm is determined for each sample using a Molecular Devices microplate reader. Cytotoxicity and antiviral efficacy of

the test compound is determined by comparison of the
absorbance values obtained in wells containing infected or
uninfected cells incubated with compounds and the
uninfected, untreated control wells.

5

HIV CULTURE PROCEDURES

STIMULATION OF DONOR LYMPHOCYTES

Buffy coats were obtained from the American Red Cross
10   or Blood Bank at Washington University School of Medicine.
These preparation are pre-screened for HIV and CMV
antibodies and HBV surface antigen (HBsAg) and ALT (alanine
transferase activity) as marker for non-A, non-B hepatitis.
Leukocyte-enriched blood (30 ml) is removed from the plastic
15   container and 15 ml is dispensed into two 50 ml screw-cap
centrifuge tubes. Each sample is diluted with an equal
volume of sterile PBS and mixed by pipeting. Ficoll-Paque
(15 ml) or LSM is placed below the diluted blood samples
using a Pasteur pipet and allowing the solution to drain to
20   the bottom of the tube. Each of the tubes is then
centrifuged at 1300 rpm (400xg) for 45 minutes at 20°C.
Following centrifugation, the lymphocyte band at the
interface is removed and transferred to a 50 ml tube.
Sterile PBS is added to dilute the separated lymphocytes and
25   then centrifuged at 1300 rpm for 8 minutes. The cell pellet
is washed two times by resuspending in PBS and
recentrifuging. The final cell pellet is resuspended in 20
ml PBS by pipetting and the total number of viable cells is
determined by Trypan Blue exclusion.

30

ACUTE INFECTIVITY ASSAYS USING CLINICAL ISOLATES

Approximately 3×10^7 cells are activated for 48 hours
with about 3-5 µg/ml PHA in RPMI continuing 10% fetal bovine
serum and IL-2 (10 U/ml). Quantitated virus stocks are
35   added to the activated lymphocyte suspension at a

multiplicity of infection of about 0.001-0.01. The cell-
virus suspension is incubated at 37°C for 2 hours to allow
virus absorption. The residual virus inoculum is removed by
centrifugation and the cells are resuspended in RPMI

5   containing 10% FBS and 10 U/ml IL-2. These infected cells
are added to the test-article diluted in complete tissue
culture medium from a stock (10 mg/ml) in DMSO in 96 well
microtiter plates to give about $5x10^5$ cells per well per
200 μl. Infected, untreated cells and cells treated with

10 DMSO alone (0.1%) or either AZT or DDI were used as
controls. The cultures were examined for syncytia formation
on days 7 and 11 post-infection or the supernants tested for
reverse transcriptase activity or p24 antigen.

15 CHRONIC INFECTIVITY ASSAYS
        CEM cells chronically infected with HXB2 (laboratory
strain of HIV-1) are added to six wells of a 12 well
microtiter plate to give about $5x10^4$ cells per well. Half
of the wells are treated with test compound at various

20 concentrations and the same number of uninfected CEM cells
are maintained without added compound. Fresh medium with or
without test compound is added each day for three
consecutive days. The cultures are then incubated for 48
hours without a change in the medium. The cells are

25 harvested by centrifugation, washed 2x in PBS and
resuspended in 50 μl 2x Laemmli buffer containing 0.125 M
Tris pH 6.8, 4% SDS, 20% glycerol, 10% beta mercaptoethanol
and 0.02% Bromophenol blue. Culture supernatants are passed
through a 0.22 μm filter to remove cell debris and the

30 centrifuged at 50,000 rpm for 90 minutes to concentrate the
virus particles. The virus pellet is resuspended in 50 μl
2x Lammli buffer. The cell or virus suspensions are boiled
for 5 minutes and then subjected to electrophoretic
separation in a 10-20% SDS-polyacrylamide gradient gel.

35 Contents of the gel are then transferred onto nitrocellulose

by electroblotting. HIV specific proteins are detected
using monoclonal antibodies to p24 and p17 followed by goat-
anti-mouse IgG linked to biotin, and avidin linked to HRP.
Enzymatic conversion of 4-chloro-1-naphthol was used to

5   visualize the specific proteins recognized by the monoclonal
antibodies. In addition, the infectivity of virus produced
by the chronically infected CEM cells in the presence or
absence or the test compounds was examined. Filtered
supernatants are serially diluted and used to infect

10 uninfected CEM cells (about 1x10⁴/well). The cultures were
examined for syncytia formation on days 7 and 11 post-
infection or the supernants tested for reverse transcriptase
activity or p24 antigen.

15 MICRO REVERSE TRANSCRIPTASE (RT) ASSAY
The micro RT assay is an adaptation of several standard RT
assays. It was developed to allow small volume quantitative
measurement of HIV RT activity and to facilitate processing
of numerous samples.

20

MATERIALS	STOCK	WORKING SOLUTION (per 1 ml)
Tris (pH 7.8)	1.0 M	50μl
25 KCI	3.0 M	25μl
DTT (store -20°C)	0.1 M	20μl
MgCl₂	0.15 M	33μl
poly (rA)p(dT012-18)	25 U/2.5ml	25μl
Pharmacia 27-7878		(0.5 U)
30 NP-40	2%	25μl
³H-TTP	2.5 Ci/ml	10μl
(NET 221A, 80 Ci/mmol)		
H₂O		777μl

35

METHOD:

1.   Add 50µl RT cocktail per well into a 96-well U-
      bottom microtiter plate.

2.   Add 10-20 µl per well of cell-free supernatant
      solution.

3.   Mix well using mechanical rotator.

4.   Incubate at 37°C for 2 hours.

5.   Aspirate onto DE81 filter paper, or equivalent,
      using TOMTEK.

6.   Rinse using 2X SSC four times.

7.   Rinse using 95% Ethanol once.

8.   Dry filter.

9.   Prepare for counting using Beta-plate counter
      (Pharmacia).

Example 19

FRESH MEDIA WITH OR WITHOUT COMPOUNDS

UNINFECTED CELLS

HIV INFECTED CELLS

SPLIT CULTURE

SEVEN DAYS

ASSAY SUPERNATANT FOR EC_{50}

SAVE CELLS FOR PCR
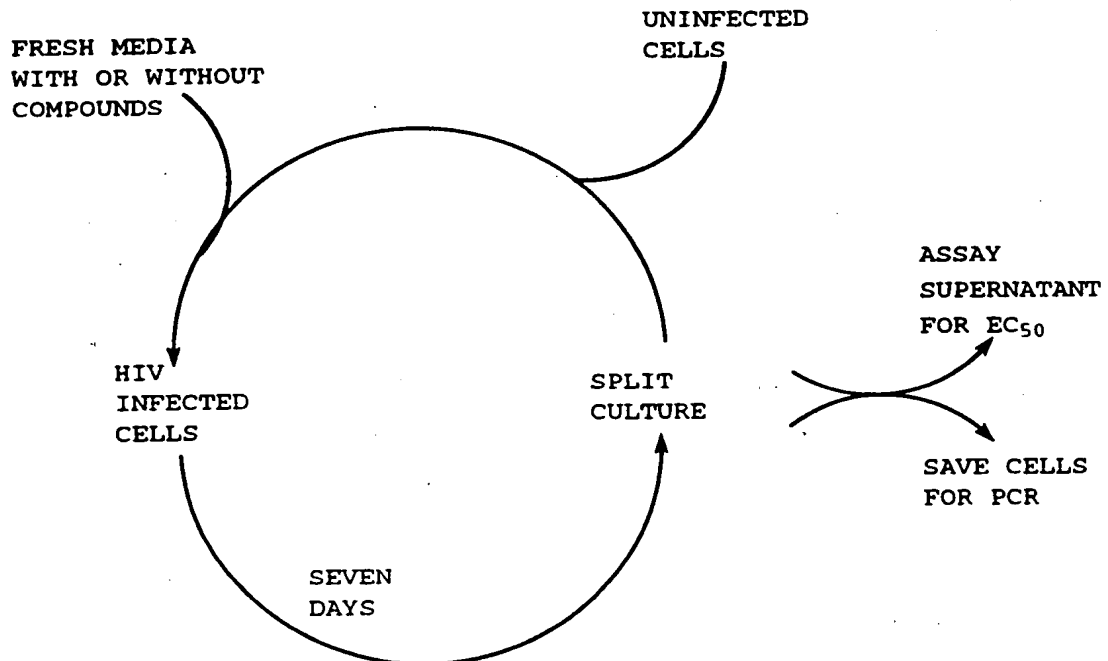
MULTIPLE CYCLES ARE REPEATED WITH INCREASING
CONCENTRATION OF COMPOUND UNTIL A SHIFT IN EC_{50} IS OBSERVED

        The following is the culture method used for selection
    of HIV protease inhibitor resistant mutants. Infected cells
    were grown continually in the presence of protease
5   inhibitor. Some cultures were subjected on alternate weeks
    to high and low inhibitor concentrations. Others were
    passaged in a constant concentration. Drug concentrations
    were increased periodically until a consistent shift in the
    EC_{50} was observed. A shift in the dose response curve was
10 generally detected at drug concentration of 0.5 to 1 μg/ml
    or greater (5-10x the EC_{90}) and depended on the viral

    isolate being treated. Both laboratory adapted and primary
    clinical isolates of HIV were used. The same virus isolates
    were passaged in the same way in the absence of drugs so
15 that direct nucleoside sequence comparisons could be made
    between treated and untreated isolates. Generally, HIV-1
    variants resistant to protease inhibitors were select by
    serial passage (growth) in the presence of several
    inhibitory concentrations of the protease inhibitor
20 specified (see Markowitz et al., Journal of Virology 69:701-
    706 (1995)). The HIV-1 variants listed below indicate the
    mutations which are present in the selected virus isolates
    and not present in the control, untreated virus isolates.

        RF represents the HIV-1 strain HIV-1$_{RF}$ and RFR represents
25 a mixture of resistant strains obtained by selection of RF
    against the compound of Example 1. RFR comprises a mixture
    of viral strains having protease genotypes of G48V (14/40
    clones); G48V, V82A (18/40 clones); G48V, L90S (2/40 clones);
    G48V, I54T, V82A (1/40 clones); G48M (1/40 clones); G48V,
30 Q61H (1/40 clones); V13I, G48V (1/40 clones); G48V, F53L,
    V82A (1/40 clones); and G48V, V82A, C95Y (1/40 clones). RFR2
    represents a mixture of resistant strains obtained by cloning
    RFR by three rounds of growth at limiting dilution. RFR2
    comprises a mixture of viral strains having protease
35 genotypes of G48V, V82A (13/15 clones); G17E, G48V, V82A

(1/15 clones); and G48V, V82A, N37D, N88D (1/15 clones).
RFRR represents a mixture of resistant strains obtained by
selection of RF against the compounds of Examples 1 and 2 and
then cloning by three rounds of growth at limiting dilution.

5   RFRR comprises a mixture of viral strains having protease
genotypes of G48V, I54T, L63P, V82A (7/9 clones); G48V, I54T,
L63P, V82A, N88S (1/9 clones); and G48V, I54T, L63P, G73M,
V82A (1/9 clones). SF162 represents the HIV-1 strain SF-162
and SF162R represents a mixture of resistant strains obtained

10   by selection of SF162 against the compound of Example 1.
SF162R comprises a mixture of viral strains having protease
genotypes of M46I, F53L, L63P, A71V, N88D (2/3 clones); and
M46I, F53L, L63P, A71V, N88D, Q92R (1/3 clones). 89-959
represents the HIV-1 strain 89-959 and 89-959R represents a

15   mixture of resistant strains obtained by selection of 89-959
against the compound of Example 2. 89-959R comprises a
mixture of viral strains having protease genotypes of N88S
(4/5 clones); and D25N, T26A, D30N, D37N, R41K, G73D, R87K,
N88S (1/4 clones). NL4 represents the HIV-1 strain HIV-1NL4-

20   3. NL4(G48V) represents a strain having a synthetically
generated site-directed mutation in the protease from glycine
to valine at amino acid position number 48. NL4(I84V)
represents a strain having a synthetically generated site-
directed mutation in the protease from isoleucine to valine

25   at amino acid position number 84. NL4(R8Q,M46I) represents a
strain having synthetically generated site-directed mutations
in the protease from arginine to glutamine at amino acid
position number 8 and methionine to isoleucine at amino acid
position number 46. NL4(P22-538) represents a mixture of

30   resistant strains obtained by selection of HIV-1NL4-3 against
the compound of Example 10 after 22 passages comprising
protease genotypes M46I, L63P, A71V, V82F, I84V (4/10); M46I,
L63P, V82F, I84V (3/10); and M46I, A71V, V82F, I84V (3/10).
NL4(P37-538) represents resistant strains obtained by

35   selection of HIV-1NL4-3 against the compound of Example 10

43

after 37 passages comprising protease genotype M46I, L63P,
A71V, I84A. NL4(538/524) represents resistant strains
obtained by selection of NL4(P22-538) against the compound of
Example 5 after 24 passages comprising protease genotype
M46I, L63P, A71V, I84A. NL4(538/P7-AG) represents a mixture
of resistant strains obtained by selection of NL4(P22-538)
against the compound of Example 12 after 7 passages
comprising protease genotypes M46I, L63P, A71V, I84A; and
V32I, V82I. NL4(538/P24-AG) represents resistant strains
obtained by selection of NL4(P22-538) against the compound of
Example 12 after 24 passages comprising protease genotype
M46I, L63P, A71V, I84A. NLA(P19-003) represents resistant
strains obtained by selection of HIV-1$_{NL4-3}$ against the
compound of Example 9 after 19 passages comprising protease
genotype R8K, M46I. NL4(P34-003) represents resistant
strains obtained by selection of HIV-1$_{NL4-3}$ against the
compound of Example 9 after 34 passages comprising protease
genotype R8K, M46I, L63P, A71V, L90M. Viral isolate
resistance results are summarized in Tables 1-11.

Example 20

The viral isolate resistance results summarized in
Tables 1-3 were generated according to the following assay
procedure or minor modifications thereof. Approximately
3×10^7 cells are activated for 48 hours with about 3-5 µg/ml
PHA in RPMI continuing 10% fetal bovine serum and IL-2 (10
U/ml). Quantitated virus stocks are added to the activated
lymphocyte suspension at a multiplicity of infection of about
0.001-0.01. The cell-virus suspension is incubated at 37°C
for 2 hours to allow virus absorption. The residual virus
inoculum is removed by centrifugation and the cells are
resuspended in RPMI containing 10% FBS and 10 U/ml IL-2.
These infected cells are added to the test-compound diluted
in complete tissue culture medium from a stock (10 mg/ml) in

DMSO in 96 well microtiter plates to give about 5×10^5 cells per well per 200 μl. Infected, untreated cells and cells treated with DMSO alone (0.1%) or either AZT or DDI were used as controls. The cultures were examined for syncytia formation on days 7 and 11 post-infection or the supernatants tested for reverse transcriptase activity or p24 antigen.

TABLE 1

Ex. No.	Virus Isolate (EC_{50} ng/mL)	
	SF162	SF162R
1	31	443
2	2	6
5	4	4
6	2	11

TABLE 2

Ex. No.	Virus Isolate (EC_{50} ng/mL)	
	SF162	SF162R
1	2	111
7	1	108
8	1	98

TABLE 3

Ex. No.	Virus Isolate (EC_{50} ng/mL)		
	89-959	89-959R	89-959R
1	96[†]	5[†]	37[‡]
2	100[†]	395[†]	872[‡]
5	36[†]	5[†]	38[‡]
6	4		6[‡]
7	13[§]	12[§]	
8	12[§]	11[§]	

[†,‡,§]Indicates values obtain in the same experiment

Example 21

The viral isolate resistance results summarized in
5   Tables 4-6 were generated according to the following assay
procedure or minor modifications thereof.   Assays are
performed in 96-well tissue culture plates.   CEM-T4 cells
are suspended in 90% RPMI media (Gibco BRL Life
Technologies, Inc., Gaithsburg, MD) 10% heat-treated, fetal
10 bovine serum (Gibco BRL Life Technologies, Inc., Gaithsburg,
MD) to a final concentration of $5x10^5$ viable cells per ml.
A frozen aliquot of an HIV culture (strain HIV-1$_{RF}$) is
thawed rapidly (in a 37°C water bath) and added to the CEM-
T4 cells to give a final concentration of about 0.001-0.01
15 infectious units per cell.   The virus-cell suspension is
rapidly mixed by swirling and 100 µL immediately added to
100 µL of each test-compound (prepared as a 2x concentrate
in 90% RPMI, 10% FBS) dilution in each well of a 96-well
tissue culture plate.   Each plate contains control wells
20 that comprise cells and virus but no test-compound.   3'-
Azido-3'-deoxythymidine (AZT) is included as a positive
control in all assays.

The tissue culture plates are incubated at 37°C in a
humidified, 5% CO_2 atmosphere for 7 days.   The level of
25 viral replication is then determined by measurement of
reverse transcriptase activity in the supernatents using
standard methods (as previously described and see, for
example, Techniques in HIV Research, Aldovini & Walker,
eds., 1990, Stockton Press, NY).

30

46

TABLE 4

Ex. No.	Virus Isolate (EC_{50} ng/mL)	
	RF	RFR
1	30	223[†]
2	40	8[†]
3	5	2[†]
4	10	0.4[†]
5	44[‡]	7[†]
6	12[‡]	15[†]

[†],[‡]Indicates values obtain in the same experiment

TABLE 5

Ex. No.	Virus Isolate (EC_{50} ng/mL)	
	RF	RFR
1	24	394
7	2	78
8	2	1

TABLE 6

Ex. No.	Virus Isolate (EC_{50} ng/mL)		
	RF	RFR2	RFRR
1	19	880	5609
2	35	367	1111
5	6	57	982
6	6	31	962
14	1	6	39
15	5	9	21
16	3	1	9

47

Example 22

The viral isolate resistance results summarized in
Tables 7-11 were generated according to the assay procedures
described by Markowitz et al., Journal of Virology, vol. 69,
701-706 (1995), which is incorporated herein by reference in
its entirety, or minor modifications thereof.

TABLE 7

Ex. No.	Virus Isolate (EC$_{90}$ nM)		
	NL4	NL4(G48V)	NL4(I84V)
5	80	160	640
6	30	150	90
10	80	160	800
12	25	125	125
13	250	1000	6250
14	8	8	72
15	60	60	60
16	8	8	8

TABLE 8

Ex. No.	Virus Isolate (EC$_{50}$ nM)	
	NL4	NL4(R8O,M46I)
5	80	240
6	30	30
10	80	240
12	25	125
13	250	750
16	8	8

48

TABLE 9

Ex. No.	Virus Isolate (EC$_{50}$ nM)		
	NL4	NL4(P22-538)	NL4(P37-538)
5	80	800	6400
6	30	150	2400
10	80	1600	6400
12	25	500	>3125
13	250	5000	>31250
14	60	60	1000
15	8	400	1000
16	8	8	40

TABLE 10

Ex. No.	Virus Isolate (EC$_{50}$ nM)			
	NL4	NL4(538/524)	NL4(538/P7-AG)	NL4(538/P24-AG)
5	80	6400	6400	6400
6	30	2400	2400	2400
10	80	6400	6400	6400
12	25	>3125	>3125	>3125
13	250	>31250	>31250	>31250
16	8	40	40	40

TABLE 11

Ex. No.	Virus Isolate (EC$_{50}$ nM)		
	NL4	NLA(P19-003)	NL4(P34-003)
5	80	240	400
6	30	30	30
10	80	400	400
12	25	150	375
13	250	1250	1250
16	8	8	8

Example 23

Protease inhibitors of Examples 1 and 2, which contain
a unique hydroxyethylurea isostere, were used to select drug
5   resistant HIV-1 variants in vitro. Clinical and laboratory
HIV-1 strains were passaged in T cell lines or peripheral
blood mononuclear cells (PBMCs) in the presence of
increasing drug concentrations. Resistant variants
consistently exhibited EC_{50} values at least 10-fold higher
10   than control virus passaged for an identical period, but in
the absence of an inhibitor. Viral DNA was amplified by PCR
and the nucleotide sequence of the gene encoding the
protease was determined using standard methods. In viruses
resistant to protease inhibitors of Examples 2 and 1,
15   respectively, an amino acid change at position 88 was
consistently observed in many of the variants selected. The
Asn residue at 88 lies within a structurally conserved
helical domain, present in both monomeric and dimeric
aspartic proteinases. The corresponding carboxy terminal
20   sequence Gly-Arg-Asp/Asn (residues 86-88) is unique to
retroviral aspartic proteinases. While any explanation for
these results is only speculation, modeling studies based on
templates derived from high resolution x-ray structures of
prototypical hydroxyethylurea inhibitors bound to
25   recombinant HIV-1 protease appear to suggest that the Asn88
mutations may alter the conformation of the protease.

Retroviral protease inhibitor compounds of the present
invention are advantageously effective antiviral compounds
30   and, in particular, are effective inhibitors of
retroviruses, particularly, lentiviruses as shown above.
Thus, the subject compounds are effective inhibitors of HIV.
It is contemplated that the subject compounds will also
inhibit other strains of HIV, such as HIV-2 and other
35   viruses such as, for example, VISNA virus and Simian

Immunodeficiency virus (SIV), HTLV-1 and HTLV-2. Thus, the
subject compounds are effective in the treatment and/or
prophylaxis of retroviral infections.

The present invention is also meant to include the
5   solvate or hydrates of the retroviral protease inhibitor
compounds, when possible, and are prepared or isolated by
methods known in the art.

The retroviral protease inhibitor compounds can be used
in the form of salts derived from inorganic or organic
10   acids. These salts include but are not limited to the
following: acetate, adipate, alginate, citrate, aspartate,
benzoate, benzenesulfonate, bisulfate, butyrate, camphorate,
camphorsulfonate, digluconate, cyclopentanepropionate,
dodecylsulfate, ethanesulfonate, glucoheptanoate,
15   glycerophosphate, hemisulfate, heptanoate, hexanoate,
fumarate, hydrochloride, hydrobromide, hydroiodide, 2-
hydroxy-ethanesulfonate, lactate, maleate, methanesulfonate,
nicotinate, 2-naphthalenesulfonate, oxalate, palmoate,
pectinate, persulfate, 3-phenylpropionate, picrate,
20   pivalate, propionate, succinate, tartrate, thiocyanate,
tosylate, mesylate and undecanoate.

Examples of acids which may be employed to form
pharmaceutically acceptable acid addition salts include such
inorganic acids as hydrochloric acid, sulphuric acid and
25   phosphoric acid and such organic acids as oxalic acid,
maleic acid, succinic acid and citric acid preferably
hydrochloride salt. Other examples include salts with
alkali metals or alkaline earth metals, such as sodium,
potassium, calcium or magnesium or with organic bases.

30   Total daily dose administered to a host in single or
divided doses may be in amount, for example, from 0.01 to 50
mg/kg body weight daily and more usually 0.1 to 30 mg.
Dosage unit compositions may contain such amounts of
submultiples thereof to make up the daily dose.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

5   The dosage regimen for treating a disease condition with the retroviral protease inhibitor compounds and/or compositions is selected in accordance with a variety of factors, including the type, age, weight, sex, diet and medical condition of the patient, the severity of the

10   disease, the route of administration, pharmacological consideration such as the activity, efficacy, pharmacokinetic and toxicology profiles of the particular compound employed, whether a drug delivery system is utilized and whether the compound is administered as part of

15   a drug combination. Thus, the dosage regimen actually employed may vary widely and therefore may deviate from the preferred dosage regimen set forth above.

The compounds of the present invention may be administered orally, parenterally, by inhalation spray,

20   rectally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. Topical administration may also involve the use of transdermal administration such as transdermal patches or iontophoresis

25   devices. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be

30   formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable siluent or solvent, for example, as a solution in

35   1,3-butanediol. Among the acceptable vehicles and solvents

that may be employed are water, Ringer's solution, and
isotonic sodium chloride solution. In addition, sterile,
fixed oils are conventionally employed as a solvent or
suspending medium. For this purpose any bland fixed oil may
5 be employed including synthetic mono- or diglycerides. In
addition, the fatty acids such as oleic acid find use in the
preparation of injectables.

Suppositories for rectal administration of the drug can
be prepared by mixing the drug with a suitable nonirritating
10 excipient such as cocoa butter and polyethylene glycols
which are solid at ordinary temperatures but liquid at the
rectal temperature and will therefore melt in the rectum and
release the drug.

Solid dosage forms for oral administration may include
15 capsules, tablets, pills, powders, and granules. In such
solid dosage forms, the active compound may be admixed with
at least on inert diluent such as sucrose, lactose or
starch. Such dosage forms may also comprise, as in normal
practice, additional substances other than inert diluents,
20 e.g., lubricating agents such as magnesium stearate. In the
case of capsules, tablets, and pills, the dosage forms may
also comprise buffering agents. Tablets and pills can
additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration may include
25 pharmaceutically acceptable emulsions, solutions,
suspensions, syrups, and elixirs containing inert diluents
commonly used in the art, such as water. Such compositions
may also comprise adjuvants, such as wetting agents,
emulsifying and suspending agents, and sweetening,
30 flavoring, and perfuming agents.

While the retroviral protease inhibitor compounds of
the present invention can be administered as the sole active
pharmaceutical agents, they can also be used in combination
with other antiviral agents which are effective against
35 retroviruses such as HIV-1. Such compounds include, but are

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not limited to, other HIV-1 protease inhibitors, various
nucleoside analogs, nonnucleoside reverse transcriptase
inhibitors, tat antagonists and glycosidase inhibitors.

Examples of HIV-1 protease inhibitors include, but not
5   limited to, Ro 31-859 (Roberts, N.A. et al. Science 1990,
248, 258-261 and Drugs of the Future 1991, 16(3), 210-212,
KNI-272, (Kagayama, S., et al. Antimicrobial Agents and
Chemotherapy 1993, 810-817), the cyclic urea series (Lam,
P., et al., "De Novo Design and Discovery of Potent,
10   Nonpeptidal HIV-1 Protease Inhibitors," paper 96 at the
205th American Chemical Society National Meeting, Medicinal
Chemistry Division, Denver, CO, March 28-April 2, 1993), L-
735,524 (Dorsey, B.D., et al., "L-735,524: The Rational
Design of a Potent and Orally Bioavailable HIV Protease
15   Inhibitor," paper 6 at the 206th American Chemical Society
National Meeting, Medicinal Chemistry Division, Chicago, IL,
August 22-27, 1993) and analogs thereof.

Examples of competitive nucleoside analogs include,
but are not limited to, azidothymidine (AZT), dideoxyinosine
20   (DDI), DDC, 3TC, D4T and PMEA. Examples of non-nucleoside,
non-competitive reverse transcriptase inhibitors include,
but are not limited to, the pyridone class (Wei, J.S., et
al. J. Med. Chem. 1993, 36, 249-255; Hoffman, J.M., et al.
J. Med. Chem. 1992, 35, 3784-3791; Saari et al. J. Med.
25   Chem. 1992, 35 3792-3802; Drugs of the Future 1992, 17(4),
283-285, and analogs thereof); the bis-
(heteroaryl)piperazines class (Romero, D.L., et al. J. Med.
Chem. 1993, 36, 1505-1508; Romero, D.L., et al. Proc. Natl.
Acad. Sci. USA 1991, 34, 746-751 and 3187-3198; and analogs
30   thereof) and the tricyclic pyridobenzo- and
depyridodiazepinones (Hargrave, K.D., J. Med. Chem. 1991,
34, 2231-2241; Merluzzi, M.J. Science 1990, 250, 1411-1413;
and analogs thereof) and 5-chloro-3-(phenylsulfonyl)indole-
2-carboxamide and its analogs (Williams, T.M. et al., J.
35   Med. Chem. 1993, 36, 1291-1294). Examples of tat

antagonists include, but are not limited to, Ro 5-3335 and
Ro 24-7429 (Hsu, M.C. et al., Proc. Natl. Acad. Sci. USA
1993, 909, 6395-6399; Tam, S. et al., "TAT INHIBITORS: A
NEW CLASS OF ANTI-HIV AGENTS," paper 372, at the 204th
5   American Chemical Society National Meeting, Organic
Chemistry Division, Washington, DC, August 23-28, 1992) and
analogs thereof. Examples of glycosidase inhibitors
include, but are not limited to, castanospermine,
castanospermine 6-butryl ester, N-butyl-1-deoxynojirimycin,
10   N-butyl-1-deoxynojirimycin per-butryl ester and analogs and
prodrugs thereof.

The therapeutic agents can be formulated as separate
compositions which are given at substantially the same time
or the therapeutic agents can be given as a single
15   compositions such that all of the active agents are at a
therapeutically effective amount in the host. Alternatively,
the therapeutic agents can be administered to the host at
different times such that only one or two active agents at a
time are at a therapeutically effective amount in the host.
20   The compounds and methods of the present invention are
effective antiviral compounds and, in particular, are
effective retroviral inhibitors as shown above. Thus, the
subject compounds are effective HIV protease inhibitors. It
is contemplated that the subject compounds will also inhibit
25   other retroviruses such as other lentiviruses in particular
other strains of HIV, e.g. HIV-2, human T-cell leukemia
virus, rous sarcoma virus, simian immunodeficiency virus,
feline leukemia virus, feline immunodeficiency virus and the
like. Thus, the subject compounds are effective in the
30   treatment and/or proplylaxis of retroviral infections.
The subject compounds and methods are also effective in
preventing the growth of retroviruses in a solution. Both
human and animal cell cultures, such as T-lymphocyte
cultures, are utilized for a variety of well known purposes,
35   such as research and diagnostic procedures including

calibrators and controls. Prior to and during the growth
and storage of a cell culture, the subject compounds may be
added to the cell culture medium at an effective
concentration to prevent the unexpected or undesired
5   replication of a retrovirus that may inadvertently or
unknowingly be present in the cell culture. The virus may
be present originally in the cell culture, for example HIV
is known to be present in human T-lymphocytes long before it
is detectable in blood, or through exposure to the virus.
10   This use of the subject compounds and methods prevent the
unknowing or inadvertent exposure of a potentially lethal
retrovirus to a researcher or clinician.

The foregoing is merely illustrative of the invention
and is not intended to limit the invention to the disclosed
15   compounds. Variations and changes which are obvious to one
skilled in the art are intended to be within the scope and
nature of the invention which are defined in the appended
claims.

From the foregoing description, one skilled in the art
20   can easily ascertain the essential characteristics of this
invention, and without departing from the spirit and scope
thereof, can make various changes and modifications of the
invention to adapt it to various usages and conditions.

WHAT IS CLAIMED IS:

1.   Method of treating retroviral infections in a
mammal comprising administering to said mammal:

5   (a)   an effective amount of a first retroviral
protease inhibitor; and
        (b)   an effective amount of a second retroviral
protease inhibitor wherein said second retroviral protease
inhibitor is effective against at least one retroviral

10   strain that is resistant to said first retroviral protease
inhibitor.

2.   Method of Claim 1 wherein said first and second
inhibitors are administered such that an effective amount of

15   both inhibitors are present in said mammal.

3.   Method of Claim 1 wherein the administration of
each said first and second inhibitors is alternated such
that an effective amount of one inhibitor at a time is

20   present in said mammal.

4.   Method of Claim 1 wherein said first retroviral
protease inhibitor is

25   N-(2(R)-Hydroxy-1(S)-indanyl)-2(R)-phenylmethyl-4(S)-
          hydroxy-5-(1-(4-(3-pyridylmethyl)-2(S)-N'-(t-
          butylcarboxamido)-piperazinyl))-pentaneamide;

          N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-
30   [[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-
          (4aR,8aS)-isoquinoline-3(S)-carboxamide;

          (2S,3R,4S,5S)-2,5-Bis-[N-[N-[[N-methyl-N-(2-
          pyridinylmethyl)amino]carbonyl]valinyl]amino]-3,4-
35   dihydroxy-1,6-diphenylhexane;

          (2S,3S,5S)-5-[N-[N-[N-methyl-N-[(2-isopropyl-4-
          thiazolyl)methyl]amino]carbonyl]valinyl]amino]-2-[N-
          [(5-thiazolyl)methoxycarbonyl]amino]-3-hydroxy-1,6-
40   diphenylhexane;

[2R-hydroxy-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]carbamic acid 3S-tetrahydrofuranyl ester;

N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-(phenylthio)-3(S)-[[N-[(2-methyl-3-hydroxyphenyl)carbonyl] amino]butyl]-(4aR,8aS)-isoquinoline-3(S)-carboxamide;

[4R-(4α,5α,6ß,7ß)]-1,3-bis[(3-aminophenyl)methyl] hexahydro-5,6-dihydroxy-4,7-bis(phenylmethyl)-2H-1,3-diazepin-2-one;

N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-2S-[[(pyrrolidin-1-yl)acetyl]amino]-3,3-dimethylbutanamide;

N-[2R-hydroxy-3-[(2-methylpropyl)[(1,3-benzodioxol-5-yl)sulfonyl]amino]-1S-(phenylmethyl)propyl]-2S-methyl-3-(methylsulfonyl)propanamide;

[1S-[1R*(R*),2S*]]-N-[2-hydroxy-3-[N^1-(2-methylpropyl)-N^1-(4-methoxyphenylsulfonyl)amino]-1-(phenylmethyl) propyl]-2-methyl-3-(methylsulfonyl)propanamide;

2S-[[(N-methylamino)acetyl]amino]-N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-3,3-dimethylbutanamide; or

(2R,3S)-3-(N-methylaminoacetyl-L-tert-butylglycinyl)amino-1-(N-isoamyl-N-(tert-butylcarbamoyl))amino-4-phenyl-2-butanol.

5.   Method of Claim 1 wherein said second retroviral protease inhibitor is

N-(2(R)-Hydroxy-1(S)-indanyl)-2(R)-phenylmethyl-4(S)-hydroxy-5-(1-(4-(3-pyridylmethyl)-2(S)-N'-(t-butylcarboxamido)-piperazinyl))-pentaneamide;

N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aR,8aS)-isoquinoline-3(S)-carboxamide;

(2S,3R,4S,5S)-2,5-Bis-[N-[N-[[N-methyl-N-(2-pyridinylmethyl)amino]carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane;

(2S,3S,5S)-5-[N-[N-[N-methyl-N-[(2-isopropyl-4-thiazolyl)methyl]amino]carbonyl]valinyl]amino]-2-[N-[(5-thiazolyl)methoxycarbonyl]amino]-3-hydroxy-1,6-diphenylhexane;

[2R-hydroxy-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]carbamic acid 3S-tetrahydrofuranyl ester;

N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-(phenylthio)-3(S)-[[N-[(2-methyl-3-hydroxyphenyl)carbonyl]amino]butyl]-(4aR,8aS)-isoquinoline-3(S)-carboxamide;

[4R-(4α,5α,6ß,7ß)]-1,3-bis[(3-aminophenyl)methyl]hexahydro-5,6-dihydroxy-4,7-bis(phenylmethyl)-2H-1,3-diazepin-2-one;

N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-2S-[[(pyrrolidin-1-yl)acetyl]amino]-3,3-dimethylbutanamide;

N-[2R-hydroxy-3-[(2-methylpropyl)[(1,3-benzodioxol-5-yl)sulfonyl]amino]-1S-(phenylmethyl)propyl]-2S-methyl-3-(methylsulfonyl)propanamide;

[1S-[1R*(R*),2S*]]-N-[2-hydroxy-3-[N^1-(2-methylpropyl)-N^1-(4-methoxyphenylsulfonyl)amino]-1-(phenylmethyl)propyl]-2-methyl-3-(methylsulfonyl)propanamide;

2S-[[(N-methylamino)acetyl]amino]-N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-3,3-dimethylbutanamide; or

(2R,3S)-3-(N-methylaminoacetyl-L-tert-butylglycinyl)amino-1-(N-isoamyl-N-(tert-butylcarbamoyl))amino-4-phenyl-2-butanol.

6.   Method of Claim 1 wherein said first retroviral protease inhibitor is

N-(2(R)-Hydroxy-1(S)-indanyl)-2(R)-phenylmethyl-4(S)-hydroxy-5-(1-(4-(3-pyridylmethyl)-2(S)-N'-(t-butylcarboxamido)-piperazinyl))-pentaneamide;

N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aR,8aS)-isoquinoline-3(S)-carboxamide;

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(2S,3R,4S,5S)-2,5-Bis-[N-[N-[[N-methyl-N-(2-
pyridinylmethyl)amino]carbonyl]valinyl]amino]-3,4-
dihydroxy-1,6-diphenylhexane;

5   (2S,3S,5S)-5-[N-[N-[N-methyl-N-[(2-isopropyl-4-
thiazolyl)methyl]amino]carbonyl]valinyl]amino]-2-[N-
[(5-thiazolyl)methoxycarbonyl]amino]-3-hydroxy-1,6-
diphenylhexane;

10   [2R-hydroxy-3-[[(4-aminophenyl)sulfonyl](2-
methylpropyl)amino]-1S-(phenylmethyl)propyl]carbamic
acid 3S-tetrahydrofuranyl ester;

N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-(phenylthio)-
15   3(S)-[[N-[(2-methyl-3-hydroxyphenyl)carbonyl]
amino]butyl]-(4aR,8aS)-isoquinoline-3(S)-carboxamide;

[4R-(4α,5α,6ß,7ß)]-1,3-bis[(3-aminophenyl)methyl]
hexahydro-5,6-dihydroxy-4,7-bis(phenylmethyl)-2H-1,3-
20   diazepin-2-one;

N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-
methylpropyl)amino]-1S-(phenylmethyl)propyl]-2S-
[[(pyrrolidin-1-yl)acetyl]amino]-3,3-dimethylbutanamide;

25   N-[2R-hydroxy-3-[(2-methylpropyl)[(1,3-benzodioxol-5-
yl)sulfonyl]amino]-1S-(phenylmethyl)propyl]-2S-methyl-
3-(methylsulfonyl)propanamide;

30   [1S-[1R*(R*),2S*]]-N-[2-hydroxy-3-[N^1-(2-methylpropyl)-
N^1-(4-methoxyphenylsulfonyl)amino]-1-(phenylmethyl)
propyl]-2-methyl-3-(methylsulfonyl)propanamide; or

(2R,3S)-3-(N-methylaminoacetyl-L-tert-
35   butylglycinyl)amino-1-(N-isoamyl-N-(tert-
butylcarbamoyl))amino-4-phenyl-2-butanol; and

said second retroviral protease inhibitor is

40   2S-[[(N-methylamino)acetyl]amino]-N-[2R-hydroxy-3-
[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)
amino]-1S-(phenylmethyl)propyl]-3,3-dimethylbutanamide.

        7.   Method of Claim 1 wherein said first retroviral
45 protease inhibitor is